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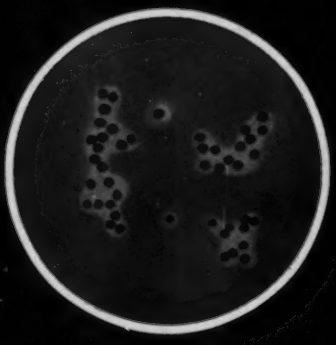

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Needle Biopsy of the Liver in Cirrhosis

Diagnostic Efficiency as Determined by Postmortem Sampling

HERBERT BRAUNSTEIN, M.D., Cincinnati

Since the work of Iversen and Roholm,[16] liver biopsy by the transcutaneous needle method has become a widely accepted diagnostic procedure. The value of this method has been demonstrated repeatedly, and it has been generally assumed that its particular applicability lies in the diagnosis of conditions diffusely affecting the liver, such as hepatitis, fatty liver, and amyloidosis. Its efficiency in the demonstration of focal lesions and those with less uniform characteristics has been questioned.*

In the fatty liver, accumulated experience indicates that the accuracy of recognition by needle biopsy approximates 100%.† This is also the case in viral hepatitis ‡ and obstructive jaundice.[37] Despite implications to the contrary, a relatively high percentage of needle specimens will reveal the presence of primary or metastatic carcinoma of the liver,§ and a similarly satisfactory yield has been noted in diseases characterized by miliary granulomas in the liver.‖

In the case of cirrhosis of the liver, it has been postulated that the reliability of the biopsy method is dependent upon the severity and uniformity of the process. In nutritional cirrhosis (portal, fatty, alcoholic) most observers have felt that a single adequate biopsy would serve to establish the diagnosis and would indicate, in a general fashion, the severity of the disease.¶ Indeed, the procurement of a specimen free of lesions or exhibiting no evidence of cirrhosis appears to be adequate evidence to exclude the existence of this condition.# Despite occasional dissenting reports,* the above-noted investigators have been able to verify this assumption by serial biopsy and/or by subsequent necropsy. In the coarser forms of hepatic fibrosis which are less widely dispersed, as in postnecrotic cirrhosis, it has been claimed that the diagnosis may be missed if the needle enters a regenerated nodule without including the cicatricial process.† It is the purpose of this presentation to evaluate these beliefs by objective means.

Materials and Methods

It was believed that an investigation by a sampling technique at the time of postmortem examination would aid in evaluating the accuracy of diagnosis of various types of portal cirrhosis by needle biopsy. Accordingly, the livers of 30 patients with one or another form of portal cirrhosis were obtained at necropsy and 18 needle specimens taken from different portions of each

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From the Department of Pathology, University of Cincinnati College of Medicine.

This investigation was carried out in part under a grant from the John R. Stark Memorial Fund.

* References 1, 5, 10, 21, 22, 25, 26, 32, 39.

† References 4, 6, 15.

‡ References 13, 16, 19.

§ References 7, 16, 28, 30, 37, 40.

‖ References 24, 25, 26, 27.

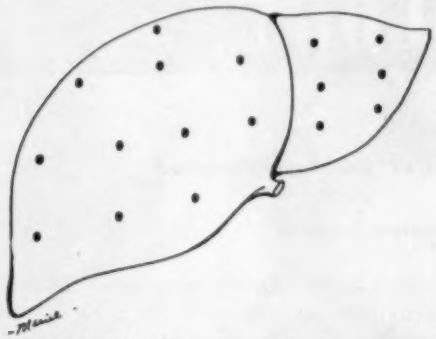
¶ References 4, 8, 14, 23, 29, 30, 34, 35, 42.

References 14, 23, 30.

* References 1, 9, 20.

† References 9, 11, 23.

liver, using the Vim-Silverman needle. The technique used has been described by Billing and associates.* It consisted of procuring 12 specimens from different, but standardized, areas in the right lobe and 6 in like fashion from the left lobe (Text Fig. 1). In occasional instances the specimens procured were unsuitable for appraisal by reason of size or artifact. These were discarded, as indicated by spaces labeled with a dagger (†) in Table 1.



Text Fig. 1.—Diagrammatic representation of anterosuperior surface of the liver. The markers indicate the sites from which the 18 needle specimens were secured.

Each adequate needle sample was classified as to the type of cirrhosis and severity of the lesion, on the basis of sections prepared in standard fashion and stained with hematoxylin and eosin and, where necessary for determination of degree of fibrosis, the Masson trichrome stain. The severity of fibrosis was indicated according to the following schema: 0, no evidence of cirrhosis; 1, moderate or early cirrhosis; 2, moderately advanced cirrhosis; 3, far-advanced cirrhosis.

The criteria utilized in estimating severity were the degree of fibrosis and distortion of lobular architecture and the amount of pseudoductular formation in the recognizable portal areas.

It was found to be relatively simple to make this estimate in the cases of nutritional cirrhosis.

TABLE 1.—*Degree and Distribution of Fibrosis**

No.	Specimen Right Lobe a b c d e f g h i j k l	Specimen Left Lobe m n o p q r	No. Valid Spec.	No. False Negatives
A. In 13 Cases of Nutritional Cirrhosis				
1	1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1	18	0
2	1 2 2 2 2 1 2 2 1 2 2 2	2 2 2 2 2 2	18	0
3	2 3 2 3 3 3 3 3 2 3 3 3	2 2 3 3 3 3	18	0
4	2 2 2 2 2 2 2 2 2 2 2 2	2 2 2 2 3 2	18	0
5	3 3 3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3	18	0
6	3 3 3 3 3 † 3 2 3 3 3 3	3 3 3 2 3 3	17	0
7	2 1 2 2 1 1 2 2 1 1 2 2	2 2 2 2 2 2	18	0
8	2 3 3 2 2 2 2 2 3 2 2 2	2 † 2 2 3 2	17	0
9	1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1	18	0
10	† 3 3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3	17	0
11	2 2 2 2 2 2 † † 2 2 † 2	1 3 2 2 2 2	15	0
12	2 1 1 1 1 † 2 † F 1 † 1	1 1 2 1 2 2	14	0
13	3 3 3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3	18	0
		Total	224	0
B. In 8 Cases of Postnecrotic Cirrhosis				
1	3 3 3 3 3 3 3 3 3 3 3 2	3 3 3 3 3 3	18	0
2	3 3 3 3 3 3 † † 3 3 † 3	3 3 1 † 3 2	14	0
3	1 1 2 2 1 3 2 2 2 2 1 1	1 1 † † † †	14	0
4	1 3 3 † 2 3 2 3 3 3 2 3	3 3 3 3 † †	15	0
5	3 3 3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3	18	0
6	1 1 1 2 2 1 1 2 1 0 1 1	1 0 1 2 0 1	18	3
7	2 3 3 2 2 0 † 3 1 1 2 2	2 3 0 2 2 2	17	2
8	2 2 3 3 2 1 3 1 2 † 2 2	2 2 2 2 2 1	17	0
		Total	131	5
C. In 9 Cases of Posthepatitic Cirrhosis				
1	1 1 1 2 1 1 1 1 1 1 1 1	1 1 1 1 1 1	18	0
2	† † 1 1 † 2 2 0 1 1 2 1	0 1 3 2 2 1	15	2
3	1 1 2 3 2 3 3 3 3 1 2 1	2 2 3 2 2 1	18	0
4	2 2 † 2 2 3 1 2 1 2 2 2	2 2 2 † 2 2	16	0
5	2 2 1 2 3 2 1 3 2 1 0 1	1 2 1 1 1 2	18	1
6	1 2 1 2 3 2 2 1 1 3 0 1	2 2 2 3 2 3	18	1
7	1 2 1 2 3 2 † 2 3 1 2 2	2 0 2 1 2 1	17	1
8	1 1 2 2 1 1 2 1 1 † † †	2 † 2 2 3 1	14	0
9	2 2 2 2 3 1 2 1 2 2 2 2	2 2 2 3 2 2	18	0
		Total	152	5

* Symbols: 0, cirrhosis could not be diagnosed; 1, early cirrhosis; 2, moderately advanced cirrhosis; 3, Far-advanced cirrhosis.
† Specimen inadequate or not secured.

Because of the irregular pattern of scarring, it proved to be considerably more difficult to appraise the status of the postnecrotic and posthepatitic forms of cirrhosis.

Definitive diagnosis and ultimate classification rested upon gross examination of the lesion at the time of autopsy and careful microscopic study of the postmortem sections procured in routine fashion. These usually consisted of two to three blocks measuring 1.5 cm. in diameter.

Classification of the type of cirrhosis was attempted in each needle sample independently. The type of cirrhosis was classified, in each case, according to the following categories:

1. *Nutritional cirrhosis* (13 cases, Table 1 A). Grossly these livers were large, yellow, micronodular and tough. This type is a pseudolobular cirrhosis usually accompanied by fatty vacuolation and, not uncommonly in the more active phase, by the presence of "alcoholic" hyalin in hepatic cells. The nodules are smaller than liver lobules. The fine fibrous scars result from abnormal extensions of connective tissue among adjacent portal areas and central veins, characteristically incorporating the latter. Bile stasis, focal necrosis, portal inflammatory exudates, and both ductular and pseudoductular proliferation may be present to a variable degree‡ (Fig. 2 *A, B, C*).

2. *Postnecrotic Cirrhosis* (8 cases, Table 1 B). This form of cirrhosis is characterized grossly by

‡ References 4, 17, 29, 31, 34, 38, 42.

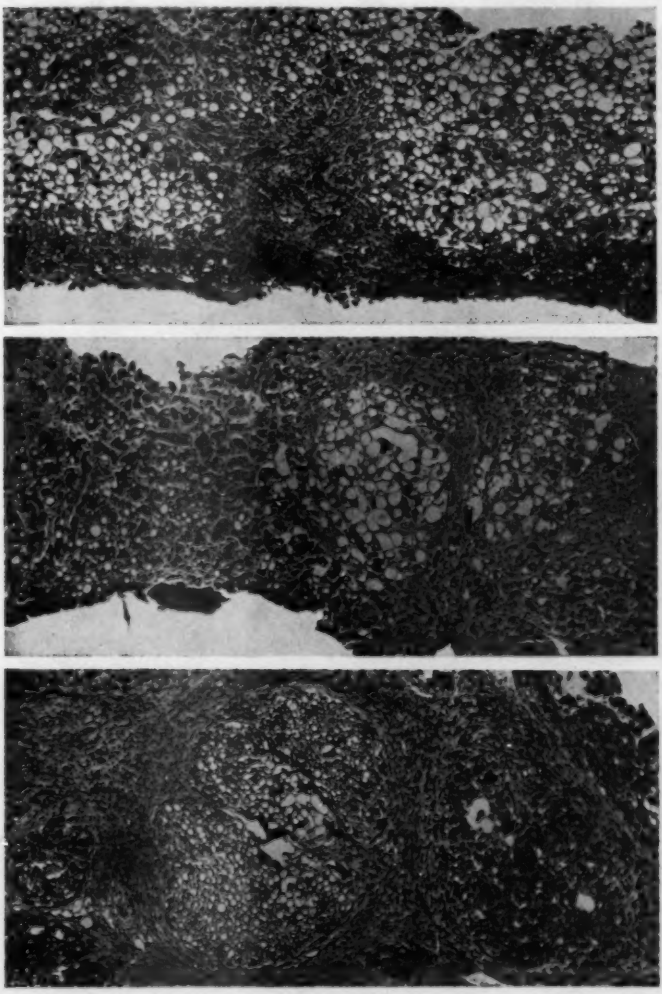
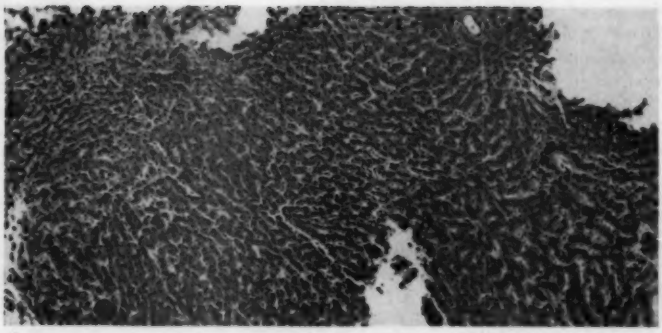
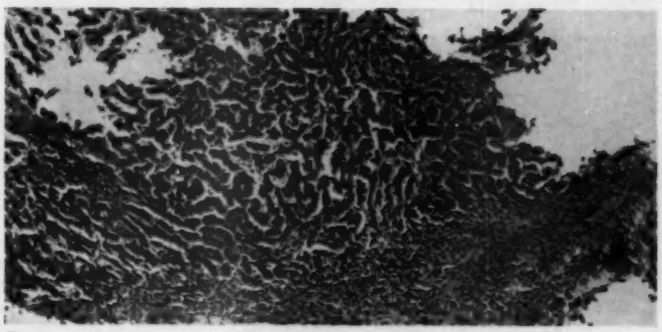


Fig. 2.—Nutritional cirrhosis. Hematoxylin-eosin stain; reduced to 3/4 of mag. × 160. *A*, Grade 1 (Case 1, Spec. a); *B*, Grade 2 (Case 4, Spec. e); *C*, Grade 3 (Case 5, Spec. g).



A



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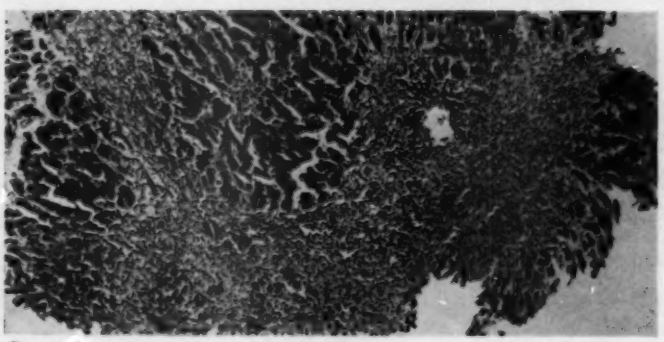


C

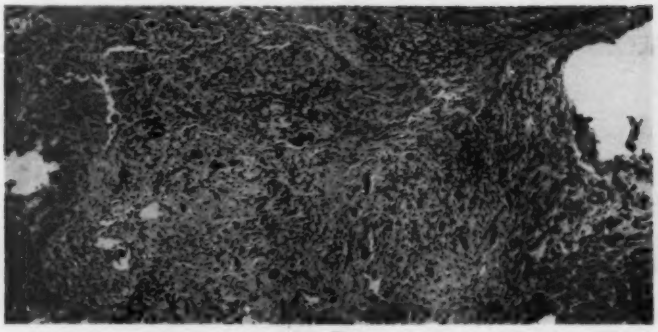
Fig. 3.—Postnecrotic cirrhosis. Hematoxylin-eosin stain.

A, 0—no evidence of cirrhosis; reduced to 3/4 of mag. × 160. Note small patches of fibrosis (Case 7, Spec. o). *B*, Grade 1 (Case 7, Spec. j); reduced to 3/4 mag. × 160; *C*, Grade 2 (Case 7, Spec. r); reduced to 3/4 of mag. × 160; *D*,

coarse scarring and by variation in size of nodules, with a range of from a millimeter to several centimeters. Microscopically, the nodules often exceed the bulk of liver lobules. Many nodules comprise large regenerating islands, but also may contain large groups of relatively unaffected lobules. Thus, in contradistinction to nutritional cirrhosis, intralobular tributaries of hepatic veins usually are manifest, although often in eccentric locations. Of particular significance is the presence of broad bands of connective tissue, representing the collagenized collapsed reticulum of many destroyed lobules consequent upon multilobular necrosis. In the trabeculae can be seen residual ductules and proliferating pseudoductules. Fat may or may not be present, depending upon the terminal nutritional status of the patient. The prominence of necrosis, inflammation, and bile stasis is also dependent upon the degree of agonal activity of the process§ (Fig. 3 *A, B, C, D, E*).

3. *Posthepatitic Cirrhosis* (9 cases, Table 1 C). This form of cirrhosis is characterized grossly by

§ References 2, 17, 21, 22, 23, 29, 31.



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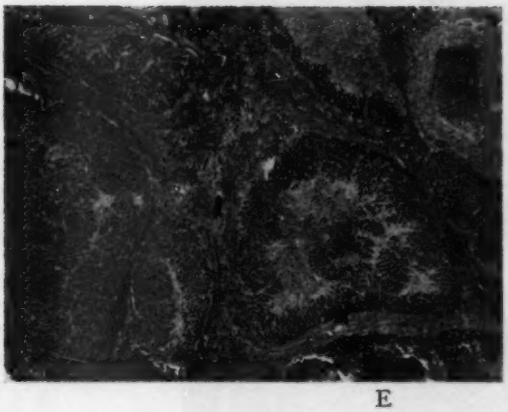


E

D, Grade 3 (Case 7, Spec. b); reduced to ¼ of mag. × 160; *E*, Case 7, postmortem 1.5 cm. section; reduced to ¼ of mag. × 80.

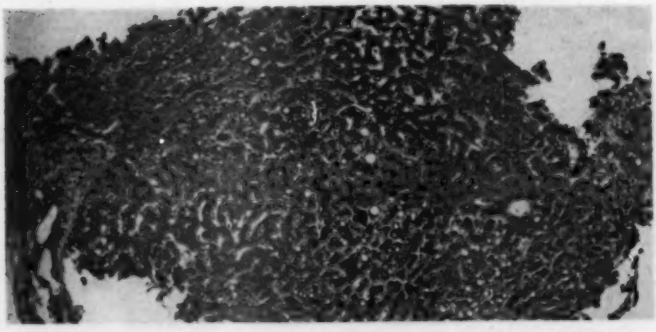
nodules intermediate in size between the nutritional and the postnecrotic form, the diameter averaging 0.5 to 1 cm. The fibrous trabeculae are relatively fine and may encompass either single lobules or groups of lobules. Each lobule retains its central vein in normal location. (Pseudolobulation, as in nutritional cirrhosis, is uncommon.) Fat is usually inconspicuous. Periportal ductular and pseudoductular proliferation may be seen. Broad scars are uncommon and reflect spotty areas of occasionally complicating submassive necrosis. The prominence of necrosis (almost invariably of individual cell type), bile stasis, and portal-area inflammation is dependent upon the degree of activity of the process‖ (Fig. 4 *A, B, C, D, E*).

‖ References 2, 3, 13, 16, 19.

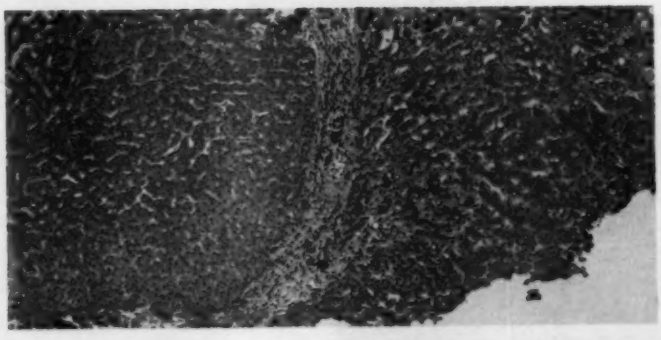
TABLE 2.—*Accuracy of Needle Biopsy in Cirrhosis of Liver*

Type Cirrhosis	No. Cases	No. Specimens	False Negatives*	Percentage Accuracy
Posthepatitic	9	152	5	96.7
Postnecrotic	8	131	5	96.2
Nutritional	13	224	0	100
Total	30	507	10	

* 10, false negatives: 6 showed foci of fibrosis; 2, badly fragmented, inadequate to rule out cirrhosis; 1, fragmented but showed focal fibrosis.



A



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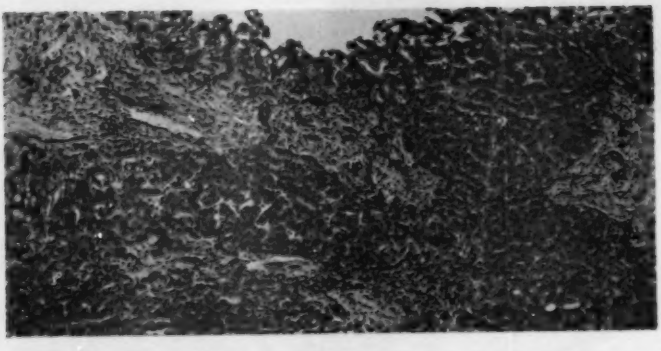

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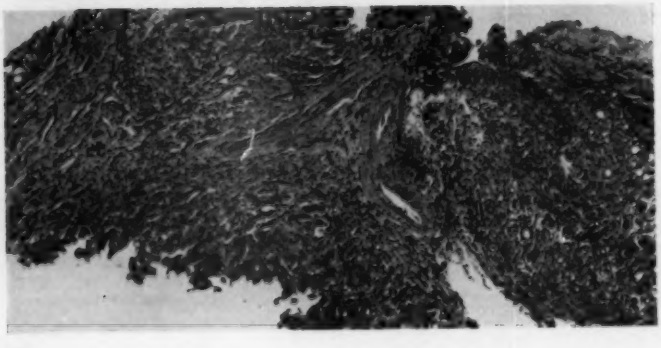
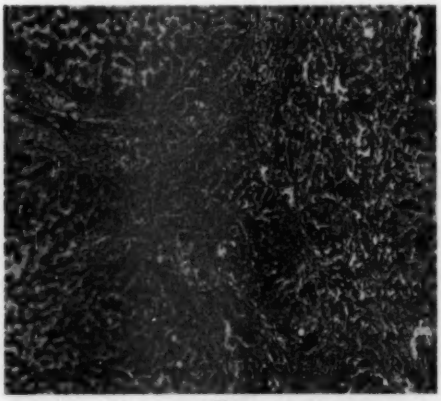


D

Fig. 4.—Posthepatitic cirrhosis. Hematoxylin-eosin stain; reduced to 3/4 of mag. × 160.

A, 0—no evidence of cirrhosis. Note area of fibrosis at one edge (Case 2, Spec. m). *B*, Grade 1 (Case 2, Spec. l); *C*, Grade 2 (Case 2, Spec. p); *D*, Grade 3 (Case 2, Spec. o); *E*, Case 2, postmortem, 1.5 cm. section.



E

Results

The results are summarized in Tables 1 and 2. In the cases of nutritional cirrhosis and postnecrotic cirrhosis, the diagnosis could be made in all adequate specimens. In posthepatitic cirrhosis, although in all cases the process was distinguishable from the nutritional form, it was frequently difficult to distinguish it from postnecrotic cirrhosis on individual samples. In three of the nine cases, even on the basis of the evidence provided by all needle samples, a definitive distinction could not be established without recourse to the information provided by the gross examination and the routine necropsy sections. The apparent reason for this is that grosser areas of necrosis complicating the posthepatitic form frequently result in scarring. Thus, the specimens traversing such scars were not distinguishable from those obtained in postnecrotic cases (Fig. 4*D*). In the estimation of the severity of fibrosis (Table 1) there is remarkable uniformity in cases of nutritional cirrhosis. On the other hand, there is considerable variation from area to area and from specimen to specimen in all but the most advanced cases of both the postnecrotic and the posthepatitic form.

Comment

Postmortem sampling techniques similar to the method used in this study have not commonly been attempted in the evaluation of the accuracy of liver biopsy. Billing and co-workers [6] used an identical method to demonstrate the distribution of fat in the liver. A similar technique, obtaining 10 specimens, was used by Waldstein and Szanto in 15 cases of various forms of liver disease.[39] They concluded that fat was uniformly distributed in the liver, while scarring, alteration of lobular pattern, and inflammation varied approximately 10% from one area to another. Gomori and Goldner,[15] using several samples from various areas of the liver in animals, demonstrated uniformity of fat distribution but some variability in distribution of glycogen. The latter finding was confirmed by Deane and associates.[12] With [43] demonstrated chemically that vitamin A is uniformly distributed in the liver. Christian [10] and Norcross and associates [27] utilized a limited sampling technique on normal and diseased livers to demonstrate uniformity in histologic appearance in various areas of the liver.

The present study demonstrates a striking accuracy in the recognition of cirrhosis by needle biopsy. All samples among the 224 procured in 13 cases of nutritional cirrhosis revealed evidence of cirrhosis. It was not possible to recognize clearly the presence of cirrhosis in only 5 of 152 specimens obtained in posthepatitic cirrhosis and in 5 of the 131 in postnecrotic cirrhosis. On the other hand, in 7 of these 10 "failures" there was some evidence of fibrosis, while 2 of the remaining 3 samples were badly fragmented, although included among the valid group.

The facility of recognition of the severity of fibrosis is apparently dependent upon the type in question. Warren and Wahi,[41] from a comparison of chemical analysis and histologic examination, maintained that reasonable accuracy could be attained in the estimation of the degree of fibrosis from a few histologic sections. As noted above, the present study appears to support the validity of

this concept when applied to the uniform scarring characteristic of nutritional cirrhosis. On the other hand, the patchy distribution of the scarring apparently renders such an assumption invalid in the postnecrotic and posthepatitic forms. The invariable monotony of the process in nutritional cirrhosis, as contrasted with the variation in pattern in the other two forms of cirrhosis, may be considered evidence that these are distinct and unrelated groups. The explanation of this phenomenon may be sought in the pathogenesis of each type. It would appear that nutritional cirrhosis develops from a progressive disorder affecting all liver cells and all areas of the liver equally. The regularity of the lesion and of its severity naturally follows. In postnecrotic cirrhosis, single or repeated episodes of sudden necrosis affect different areas of the liver in an unpredictable, irregular fashion. In the lesion classified as posthepatitic cirrhosis, the inflammatory process is apparently uniformly distributed but largely localized to portal areas. The fibrosis appears to vary to a certain extent from portal area to portal area. The occasional superimposition of foci of multilobular necrosis presumably leads to spottily distributed broad scars, simulating in these areas postnecrotic cirrhosis. Both of these factors contribute to lack of uniformity of the process.

Summary and Conclusions

A sampling technique, using the Vim-Silverman needle, was utilized to determine the accuracy of liver biopsy in the diagnosis and estimation of the degree of severity of cirrhosis of the liver.

Of 507 adequate samples, only 10 failed to reveal cirrhosis.

In cases of nutritional cirrhosis the accuracy of diagnosis was 100%. The degree of the fibrosis revealed a remarkable uniformity in all areas of the liver.

In cases of posthepatitic and postnecrotic cirrhosis the accuracy of recognition of the presence of cirrhosis was roughly 96%. In many of the falsely negative samples some fibrosis was present.

In the above two forms of cirrhosis, there was considerable variation in the intensity of fibrosis, and the biopsy method would therefore be of little value in the appraisal of severity.

It is concluded that needle liver biopsy represents a singularly accurate method of diagnosing cirrhosis of all three types. A liver biopsy which fails to demonstrate cirrhosis or fibrosis rules out cirrhosis with reasonable certainty. As a means of estimation of the severity of the process and following its progression or regression, accuracy may be assumed only in the case of nutritional cirrhosis.

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Responses of the Liver to Injury

Effect of Previous Injury upon the Healing Pattern After Acute Carbon Tetrachloride Poisoning

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Introduction

This report deals with some of the long-term effects of an acute hepatic injury. In earlier investigations it was found that while the centrilobular necrosis produced by a single injection of carbon tetrachloride (CCl_4) healed within five days, marked nuclear polyploidy was found eight weeks later.[1] These observations suggested that the liver, which now had a new type of nuclear population, might be altered biologically. The present experiments were designed to test this hypothesis by subjecting the liver to a second injury. The results show that the response to the second injury is altered and that healing is delayed.

Material and Method

The design of the experiments was to produce an hepatic injury with carbon tetrachloride, as described earlier,[1] and after an interval of eight weeks to produce a second injury with carbon tetrachloride. The course of healing of this second injury was compared with that of a single injury in animals of similar stock, age, sex, and dietary regimen.

Male rats, obtained from the Wistar Institute in Philadelphia, were employed throughout. They were caged individually and were fed Purina Checkers.

Group A.—These 10 animals were killed at 16 weeks of age and did not receive any CCl_4. They served as age and diet controls for those rats which received one hepatic injury at 16 weeks of age.

Group B.—These 11 animals received a CCl_4 injury at 8 weeks of age and were killed when 16 weeks old. They served as controls for those rats which had two hepatic injuries, at 8 and 16 weeks of age.

Group C.—These 26 animals received a CCl_4 injury at 16 weeks of age and were killed 48, 72, and 120 hours later. Eight, eight, and ten rats were killed at the respective times after CCl_4. These animals served as controls for animals which received two hepatic injuries.

Group D.—These 27 animals received one CCl_4 injury at 8 weeks of age and the second at 16 weeks of age. They were killed 48, 72, and 120 hours later. Ten, nine, and eight rats were killed at the respective times after CCl_4.

The livers were examined histologically, cytochemically, and chemically by methods described elsewhere.[1]

Results

All animals thrived and survived the procedure.

Histological.—Group A.—No CCl_4 (Fig. 1).

All of the livers were normal.

Group B.—CCl_4 at 8 weeks of age (Fig. 1).

All of the livers were normal except for the presence of some binucleated liver cells.

Group C.—CCl_4 at 16 weeks of age.

The animals in this group showed changes similar to those described elsewhere in 8-week-old rats of the same sex and stock which were given CCl_4 and were studied serially.[1]

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From the Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, and the Department of Zoology, Columbia University.

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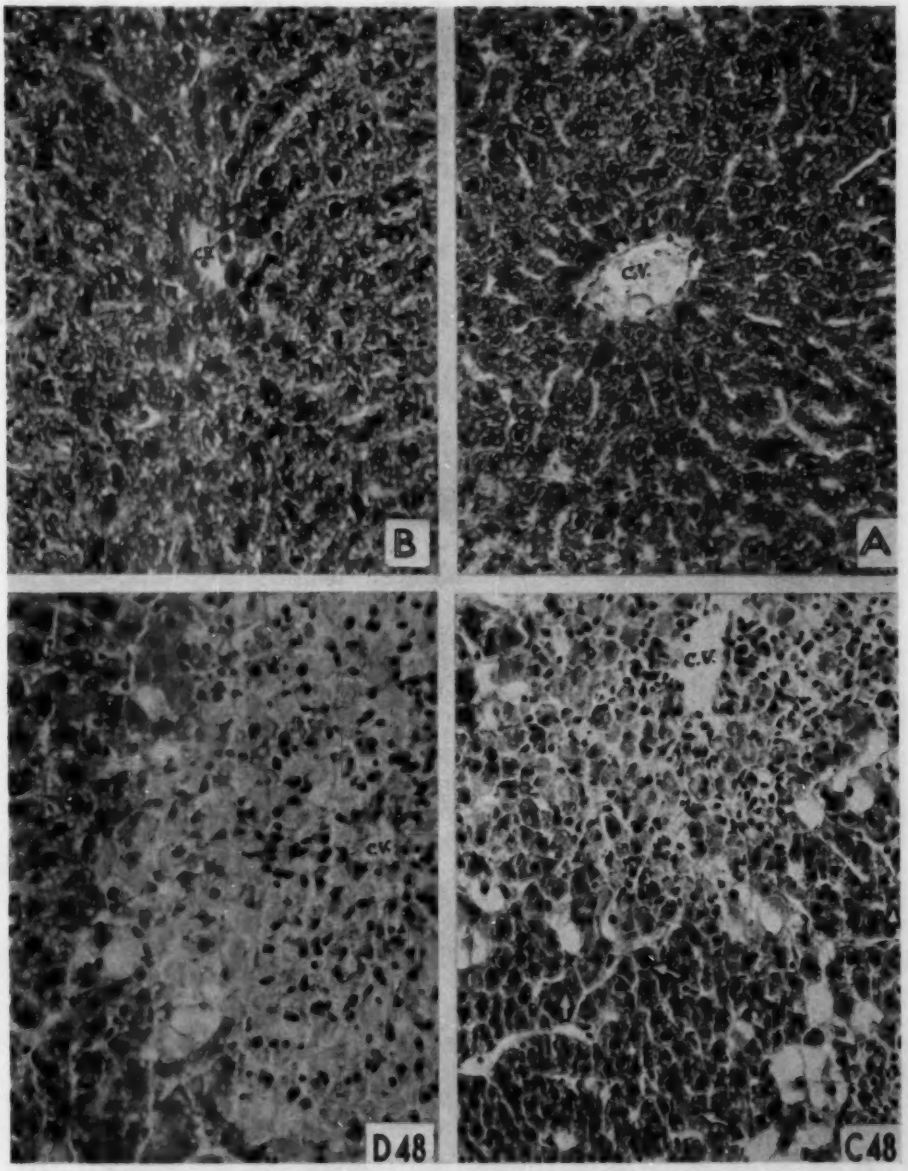

Fig. 1.—Group A: No CCl_4. *C.V.*, central vein. The liver is normal.

Group B: CCl_4 at 8 weeks of age. The liver is normal except for the presence of some binucleated liver cells.

Group C48: CCl_4 at 16 weeks of age. Rat killed after 48 hours. Marked centrilobular necrosis with inflammatory infiltration is seen. Four mitotic figures are noted (arrows) in the surviving cells.

Group D48: CCl_4 at 8 and 16 weeks of age. Rat killed 48 hours after second injection. Marked centrilobular necrosis with some inflammatory infiltration is seen. No mitoses are seen in the surviving cells.

Giemsa stain; reduced to 80% of × 250.

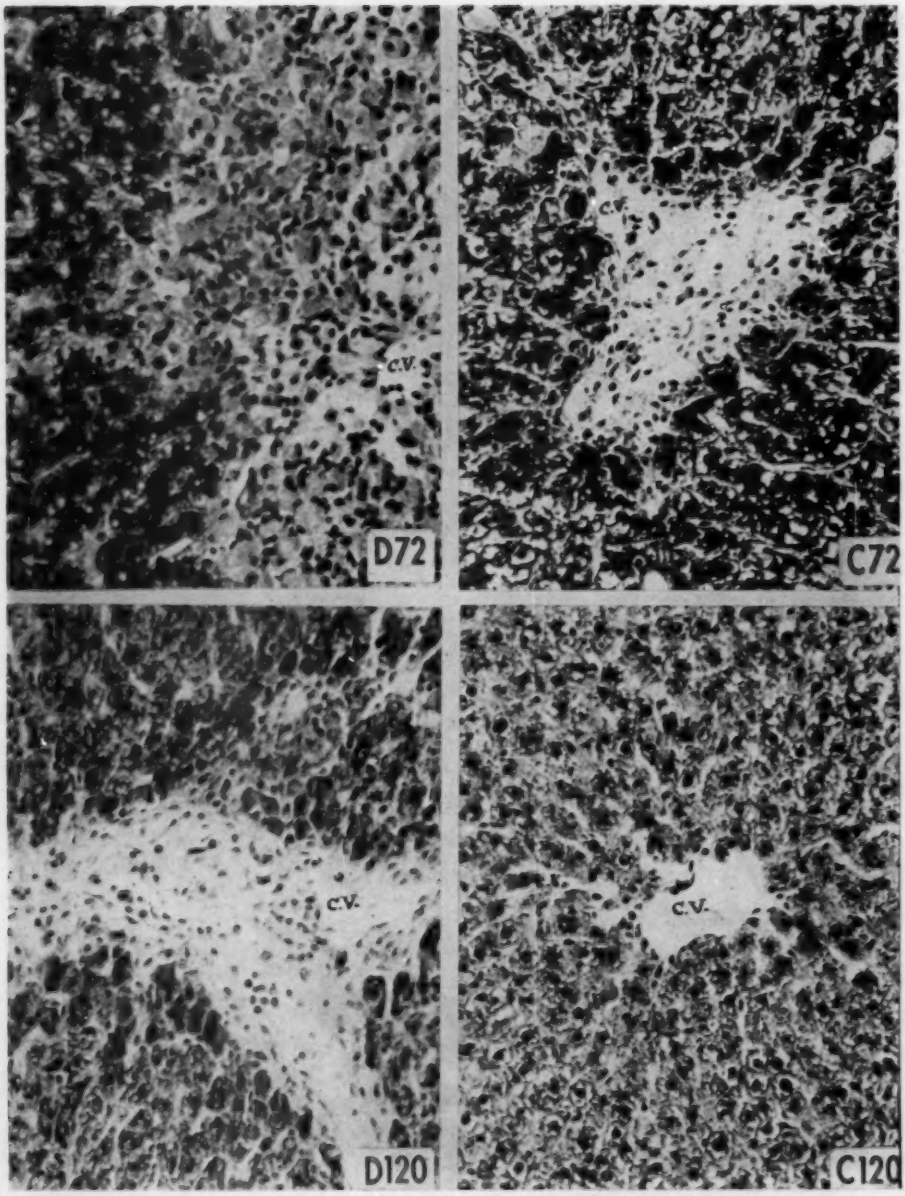

Fig. 2.—Group C72: CCl_4 at 16 weeks of age. Killed after 72 hours. The necrotic cells have disappeared, and loose reticulum and new liver cells occupy the pericentral zone.

Group C120: Killed after 120 hours. The liver is normal except for some binucleated liver cells.

Group D72: CCl_4 at 8 and 16 weeks of age. Killed 72 hours after second injection. Similar to C48. Two mitotic figures are noted (arrows) in the surviving cells.

Group D120: Killed after 120 hours. Similar to C72.

Giemsa stain; reduced to 80% of × 250.

Forty-Eight Hours (Fig. 1): There was centrilobular necrosis involving one-third to one-half of the lobular radius, and this area was infiltrated with mononuclear and polymorphonuclear elements. Peripheral to this zone were surviving liver cells containing large to small vacuoles. In the periportal zone the liver cells showed two to three mitotic figures per high-power field.

Seventy-Two Hours (Fig. 2): The necrotic cells were cleared and the central area was occupied by a latticework of reticulum with small liver cells filling the adjacent area. Only occasional mitoses were seen.

One Hundred Twenty Hours (Fig. 2): The livers were normal except for the presence of some binucleated liver cells.

GROUP D.—CCl_4 at 8 and 14 weeks of age.

Forty-Eight Hours (Fig. 1): The livers showed only occasional mitoses but otherwise were similar to those of Group C 48 hours after CCl_4.

Seventy-Two Hours (Fig. 2): The livers showed three to four mitoses per high-power field in the surviving cells, but otherwise they were similar to those in this same group 48 hours after CCl_4. When compared with Group C, they were similar to animals 48 hours after CCl_4.

One Hundred Twenty Hours (Fig. 2): The livers appeared similar to those of Group C at 72 hours after CCl_4.

Spectrophotometric-Cytochemical. — The studies of the frequency distribution of nuclear classes according to their deoxyribonucleic acid (DNA) contents (Table) may be summarized as follows:

1. The frequency distribution in the 16-week-old animals, which had never received CCl_4, was the same as that reported elsewhere for 8-week-old animals.[1]

2. Eight weeks after a CCl_4 injury a shift to higher classes persisted. This confirmed the observations made earlier.[1]

3. At 48 hours after CCl_4 maximal polyploidy was present in the livers of rats which had received one injury (Group C). On the other hand, the livers of those rats which had received the second CCl_4 injury (Group D) showed little change in nuclear ploidy at this time when compared with their respective controls. (Group B).

4. At 72 hours after CCl_4, when the liver nuclei of the single injury group (C) had begun to return toward the preinjury distribution pattern, maximal polyploidy was observed in those animals which had received a second CCl_4 injury (Group D). The level of polyploidy achieved by this latter group at 72 hours was similar to that observed in Group C at 48 hours.

5. At 120 hours after CCl_4 nuclear polyploidy was present in Groups C and D. In each instance the number of octaploids exceeded that found eight weeks after CCl_4 (Group B).

Chemical.—The livers of rats after one injury (Group C) showed smaller increments in liver weight than did those of rats after two injuries (Group D).

For the evaluation of the changes in nucleic acids (dry weight) in Groups C and D, each was compared with its respective control. Group A was the baseline for Group C and Group B for Group D. The nucleic acid levels in Group B were significantly higher than those of Group A.

The maximal increment in Group C occurred 48 hours after CCl_4, when DNAP and RNAP levels rose 95% and 58%, respectively. Thereafter there was some decline in nucleic acids levels, but at 120 hours DNAP and RNAP were 82% and 37% above their preinjury levels.

On the other hand, in Group D, which had received two hepatic injuries, at 48 hours after the second injury, DNAP and RNAP rose only 31% and 10%, respectively. The maximal increase occurred at 72 hours when DNAP and RNAP were elevated by 62% and 42%, respectively. At 120 hours there was some decline from the peak levels to increments of 48% and 33% for DNAP and RNAP, respectively.

The nitrogen levels (dry weight) of the several groups were not altered significantly during the progress of the injury.

Summary of Analytical Data

Groups	No. Rats	Liver			DNAP		RNAP		Nitrogen	Frequency Distribution of Nuclear Classes (Per Cent of Group)		
		Wt., Gm.	Per Cent Body Wt.	Per Cent* Dry Wt.	Dry Wt. γ/Mg.	SEM	Dry Wt. γ/Mg.	SEM	Dry Wt. Mg/Mg.	2X	4X	8X
A–16 wk.–No CCl_4	10	9.95	3.24	21.9	0.87	0.01	4.10	0.06	0.125	20	78	2
B–16 wk.–CCl_4 at 8 wk.	11	10.65	3.37	20.7	1.08	0.03	4.60	0.10	0.132	4	90	7
C–CCl_4 at 16 wk.												
48 hr. after CCl_4	8	10.72	4.07	14.8	1.70	0.05	6.49	0.11	0.136	1	75	24
72 hr. after CCl_4	8	11.81	4.03	15.2	1.58	0.06	6.53	0.24	0.134	2	89	9
120 hr. after CCl_4	10	10.31	3.41	17.6	1.58	0.05	5.63	0.16	0.136	1	86	13
D–CCl_4 at 8, 16 wk.												
48 hr. after CCl_4	10	12.14	4.04	16.4	1.41	0.06	5.05	0.09	0.132	2	88	11
72 hr. after CCl_4	9	14.19	4.65	15.7	1.75	0.06	6.63	0.14	0.137	0	77	24
120 hr. after CCl_4	8	10.12	3.64	20.7	1.60	0.09	6.13	0.25	0.139	1	82	18

* This figure represents the residual dry weight after cold trichloroacetic acid (TCA) and ether-alcohol extractions.[1]

The histological, cytochemical, and chemical data were in good agreement and may be summarized as follows:

1. Eight weeks after one injection of CCl_4 the liver appeared normal histologically but showed some binucleated cells. At this time nuclear polyploidy and tissue DNAP and RNAP levels were greater than those observed in the livers of rats of the same age which had never been subjected to injury.

2. In the livers which had been injured once, the peak of mitotic activity, nuclear polyploidy, and nucleic acid levels occurred 48 hours after injury. By 120 hours cytoarchitectural restoration was completed, and some binucleated liver cells were present. The degree of nuclear polyploidy and the levels of tissue nucleic acids, while less than 48 hours, were still above their preinjury levels.

On the other hand, in the livers which had been injured twice, the peak of mitotic activity, nuclear polyploidy, and nucleic acid levels occurred 72 hours after the second injury. By 120 hours, cytoarchitectural restoration was not completed, but had reached the stage found at 72 hours in the singly injured livers. In the former group the tissue nucleic acid increments were less than those of the latter group.

Thus, from the evidence derived by three techniques, the histological, the cytochemical, and the chemical, healing was delayed significantly following the second injury.

Comment

The results of the experiments show that the liver of the rat, injured but once with CCl_4 and allowed to recover, remains altered long after the first injury. One manifestation of this altered biological state is the delayed healing which occurs after a second injury.

These findings indicate that the conventional criterion for healing of the liver, namely, cytoarchitectural restoration, does not give a true estimate of the biological state of the organ. A clue that these livers might be changed came from the nuclear polyploidy observed after the first injury.[1] It would be an oversimplification to ascribe this altered healing response, in itself a most complex metabolic phenomenon, solely to the nuclear polyploidy. It seems more likely that nuclear polyploidy is but one characteristic of these altered cells and marked biological change is associated therewith.

The data indicate that after injury and stimulus to regeneration some of the surviving cells double their nuclear DNA in preparation for cell division. Of this group, many divide and revert to their original class. However, some remain undivided. In this manner the nuclear population type changes. Experiments are in progress to observe whether, with successive hepatic injuries, separated by intervals sufficient for histological restoration, more marked residual nuclear polyploidy may be induced, and, along with this, a progressive

lengthening in the time required for healing. Thus far, four injuries have been studied. The data on the histological healing are incomplete. However, studies of the nuclear classes show that, whereas the normal rat liver may have about 2% octaploids, after the first injury there are about 7%-9% octaploids. This increases to 15%-17% after the second injury and remains at this level even after the fourth injury. This type of polyploidy differs from that which accompanies thioacetamide feeding. In the latter case nuclear classes revert to normal after withdrawal of thioacetamide.*

The concept of injury as a precursor to cancerous transformation is an old one. Repeated injury of the liver with CCl_4, without healing between injuries, produces hepatic cirrhosis [4] and eventually hepatic cancer.[5] Furthermore, it is known that primary liver cancer in man is seen more frequently in livers which have been the seat of cirrhosis than in normal livers.[6]

What may be an important chain of evidence in this transformation of normal cells to cancer cells has been reported recently. Bader [7] found that in the transition from normal to precancerous metaplasia to cancer of the rat prostate, induced by methylcholanthrene, there occurs a progressive increase in nuclear polyploidy, which becomes most marked in the cancerous tissue. Leuchtenberger and associates [8] observed nuclear polyploidy in human tumors.

While much remains to be learned in this very complex area, it is possible that with repeated stimuli to regeneration, whether induced by tissue injury or by humoral or other mechanisms, there may occur a gradual transformation of nuclear types along with associated cellular metabolic changes, which eventuate in a cellular population with altered growth potentials.

The present findings are of considerable speculative interest in relation to the genesis of chronic liver disease. Since a single injury produces such profound long-range effects upon healing, then the capacity of the organ to recover from subsequent injuries may become increasingly impaired. Eventually, this may interfere with the normal "wear and tear" replacement of liver cells, so that the rate of regeneration lags behind the rate of degeneration. Such a sequence of events might account for the chronic liver lesions which persist, and sometimes progress, long after the initiating offending factors have disappeared. Inflammatory-cell infiltration and hepatic fibrosis may continue in the livers of patients with Laennec's cirrhosis long after the patients have practiced abstention from alcohol, and have maintained good nutritional and clinical states. The same may occur in the livers of persons who have had viral hepatitis. Further, while little is known about the normal life span of liver cells, it is possible that, along with the post-injury change in regenerative capacity, there may be a shortening in the life span of the cells. These phenomena could eventuate in the profound anatomic and functional derangements which occur in many patients.

* References 2 and 3.

Summary

1. A marked change in hepatic-cell nuclear type remains long after histological repair of the acute CCl_4 injury to the liver.

2. The response of such a liver to a second injury is characterized by a significant delay in healing.

3. Some theoretical implications of these findings are suggested.

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Mycotic Endocarditis of the Tricuspid Valve Due to Aspergillus Flavus

Report of a Case

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and
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A recent autopsy disclosed a large vegetation of the tricuspid valve which was composed predominantly of mycelial elements. These were identified as Aspergillus flavus by culture. This paper records the clinical and morphologic details of the case and discusses some of the mechanisms by which fungi, ordinarily nonpathogenic for man, may cause fatal infections.

Report of Case

Clinical History.—The patient, a 50-year-old white man, was admitted to Charity Hospital of Louisiana at New Orleans on Dec. 29, 1954, complaining of weakness and intermittent fever. During the previous two years he had been treated elsewhere for severe anemia and had received iron compounds, intermittent corticotropin, and 26 blood transfusions. A tentative diagnosis of chronic lymphocytic leukemia had been considered on the basis of several bone marrow aspirations that showed hypoplastic marrow containing many mature lymphocytes.

On admission a soft liver was palpable 5 cm. below the right costal margin. The spleen extended 9 cm. below the left costal margin. Moderately enlarged lymph nodes were present in the axillary, supraclavicular, and inguinal regions.

Laboratory findings on admission were as follows: hemoglobin, 6.8 gm. per 100 cc.; hematocrit, 19 vol. per 100 cc.; red blood count, 1,770,000 per cubic millimeter; white blood count, 850 per cubic millimeter; differential blood count, 40% lymphocytes, 30% neutrophiles, 12% band forms, 16% myelocytes, 1% eosinophiles, and 1% basophiles; platelets, 119,000 per cubic millimeter; bleeding time, 4.5 minutes; coagulation time (Lee and White), 6 minutes; erythrocyte sedimentation rate, 76; urinalysis, negative.

Bone-marrow aspiration was attempted, but no marrow was obtained. On Jan. 11, 1955, a surgical biopsy of the sternal marrow was performed, and this revealed the presence of many normoblasts and a heavy infiltration of the marrow with small mature lymphocytes. The findings suggested chronic lymphatic leukemia with a secondary hemolytic anemia, possibly associated with hypersplenism. A needle biopsy of the liver revealed diffuse deposits of hemosiderin pigment but no extramedullary hematopoiesis.

It was felt that splenectomy was indicated, but a staphylococcic infection at the site of the sternal biopsy developed and the operation was delayed. The patient was treated with 1 gm. of oxytetracycline, 250 mg. of tetracycline, and 30 units of corticotropin per day. After 45 days of this regimen and 19 blood transfusions, a splenectomy was performed on March 25, 1955. The immediate postoperative course was satisfactory. Thirteen days after surgery, however, the patient became dyspneic and much weaker. X-ray of the chest revealed a pneumonic infiltrate of the right upper lobe and a small amount of fluid in the right pleural cavity. He was given increased doses of penicillin, streptomycin, and tetracycline, in addition to 20 units of corticotropin daily. Three days later, on the 16th day after splenectomy, the patient developed sudden peripheral vascular collapse and died the following day.

Histologic examination of the spleen revealed numerous deposits of hemosiderin but no extramedullary hematopoiesis. There were many small areas of granulomatous inflammation, in which no organisms could be identified.

Autopsy Findings.—The major findings were limited to the heart, lungs, mediastinal lymph nodes, and bone marrow.

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Present Address: Clinical Center, National Institutes of Health, U. S. Public Health Service (Dr. Kirschstein).

The heart weighed 350 gm. Upon opening the right ventricle and pulmonary artery, a large, firm, reddish-brown polypoid mass was encountered, which measured 7×4×3 cm. The mass was firmly attached to the inferior surfaces of all three leaflets of the tricuspid valve, which was virtually destroyed (Fig. 1). On section, the mass was seen to consist of dry, grayish-pink strands of friable material. The region of attachment to the tricuspid valve and

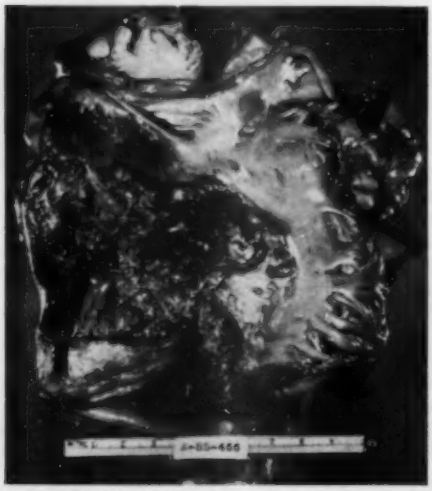

Fig. 1.—Right auricle, tricuspid valve, and right ventricle. Note large vegetation involving tricuspid valve.

the adjacent ventricular myocardium was yellow and necrotic. Fibrinopurulent material exuded from this region of the myocardium.

The lungs weighed 1400 gm. together. Both lower lobes were firm, dark red, and covered on their pleural surfaces by rough fibrinous exudate.

The mediastinal and tracheobronchial lymph nodes were enlarged and matted together. Most of them were caseous on section.

Bone marrow, taken from multiple sites, was red and appeared to be hyperplastic.

Other significant gross findings included thrombosis of the portal vein at its bifurcation into right and left branches and of the splenic artery and hemorrhage into the tail of the pancreas.

Microscopic study showed that the vegetation consisted almost entirely of branching hyphae and spores of a fungus (Fig. 2). In many areas the hyphae had penetrated deeply into the myocardium, and here the muscle was necrotic and infiltrated by neutrophilic leucocytes (Fig. 3). In several regions adjacent to the tricuspid ring, fungi had penetrated completely through the myocardium and were accompanied by an inflammatory reaction extending to the epicardial surface.

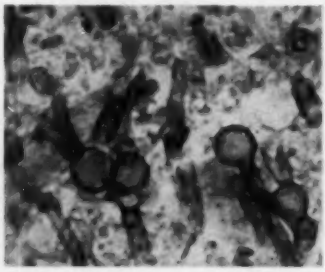

Fig. 2.—Tissue section showing chlamydospores. High-power magnification; Gridley stain.

Fig. 3.—Myocarditis, with demonstration of direct invasion by fungi. Gridley stain.

The visceral pleura of the lower lobe of both lungs was thickened. This thickening was composed of fibrin and collagen deposits, among which were aggregates of septate hyphae surrounded by lymphocytes, macrophages, and polymorphonuclear leucocytes. Throughout the lung substance there were small and large granulomas, consisting of epithelioid cells, giant cells, lymphocytes, and areas of necrosis. In only one granuloma were fungi identified. However, many of the veins and arteries contained masses of hyphae occluding their lumens and extending into their walls (Fig. 4).

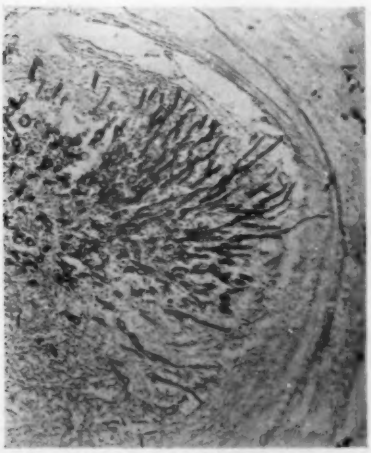

Fig. 4.—Involvement of pulmonary vessel by fungi. Photomicrograph demonstrates septate hyphae and chlamydospores. Gridley stain.

The fungi stained poorly by the periodic acid-Schiff method but were brought out clearly with the Gridley and Bauer stains. The hyphae were large, septate, and in chains. Many round, thick-walled chlamydospores were present. These histologic findings suggested that the fungus was probably a member of the genus Aspergillus. Cultures of the endocardial vegetation and of caseous lymph nodes yielded yellow-green colonies on Sabouraud's agar. The conidiophores on microscopic examination revealed pitted walls. According to Gilman,[1] these findings lead to the identification of A. flavus.

No fungi were identified in sections of the caseous mediastinal and tracheobronchial lymph nodes.

The sections and smears of the bone marrow revealed hyperplasia of all elements with areas of mature lymphocytes. No abnormal forms were identified, and no granulomas were found. In spite of the clinical and hematological findings that suggested the diagnosis of chronic lymphocytic leukemia, no unequivocal evidence at autopsy substantiated this diagnosis. In spite of the nests of mature lymphocytes in the marrow, it seemed unlikely that leukemia was present.

The portal vein contained a partially organized thrombus.

Comment

Three cases of endocarditis caused by Aspergillus have previously been reported. Zimmerman's[2] two cases were diagnosed histologically without cultures. Welsh and Buchness[3] reported one case confirmed by culture. In two of these cases[2] the vegetations were large, and each measured up to 2.5 cm. in diameter. In one case the aortic valve and in the second case both aortic and tricuspid valves were involved. In the third case[3] there was a velvety yellow plaque, 2 cm. in diameter, on the endocardium of the right ventricle. In our case a 7×4×3 cm. polypoid vegetation attached to the tricuspid valve had propagated and partially obstructed the outflow tract of the right ventricle.

Zimmerman[4] recently reviewed the literature and stressed the increasing frequency of fungus infections as serious complications of other diseases. He attributed the invasion, localization, and dissemination of these organisms to a combination of three factors. First, there appears to be an increased susceptibility to fungus infections in patients with any debilitating disorder. Secondly, the presence of a local lesion of the skin or mucous membranes may serve as a portal of entry. Thirdly, an ecologic disturbance between fungi and bacteria may result from various therapeutic combinations of antibiotics and hormones. The suppression of bacterial growth by broad-spectrum antibiotic treatment combined with the suppression of the inflammatory response by corticotropin and cortisone may produce an environment favorable to the growth of normally nonpathogenic fungi. Our patient was a debilitated man with severe pancytopenia who was treated by multiple broad-spectrum antibiotics and corticotropin. The portal of entry for the fungus may have been the sternal wound, but more probably was the respiratory tract. It is of interest that in both our

case and the one reported by Welsh and Buchness [3] splenectomy was performed because of leucopenia. Both patients were treated with numerous broad-spectrum antibiotics and corticotropin. The two patients reported by Zimmerman [2] had also received large doses of antibiotics. The anti-inflammatory properties of cortisone and corticotropin are well documented,[5] as is the development of certain fungus infections during broad-spectrum antibiotic treatment.[6] In our case and in those of Zimmerman and of Welsh and Buchness one or both of these factors were contributory. These findings suggest that the administration of anti-inflammatory hormones and broad-spectrum antibiotics may lead to conditions favorable to fatal dissemination and growth of fungi which are not ordinarily pathogenic for man.

Summary

A case of endocarditis of the tricuspid valve caused by Aspergillus flavus is reported. The history of this case and of three others from the literature suggests that ordinarily nonpathogenic fungi may produce fatal infections in debilitated patients who have been given massive doses of broad-spectrum antibiotics. The possibility that anti-inflammatory hormones, such as cortisone, play a role in the pathogenesis of such infections is considered.

Species identification was performed by Dr. John D. Schneidau Jr., of the Department of Microbiology, Tulane University of Louisiana School of Medicine.

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A Carotid-Body-like Tumor on the Left Subclavian Artery

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The purpose of this paper is to report a case of a carotid-body-like tumor occurring high on the left subclavian artery of a human male. From its location and its morphologic characteristics, it is considered to represent a tumor most probably arising from an aortic body, and is unusual for at least two reasons. First, aortic body tumors in humans are so uncommon that only two examples have previously been reported. Second, the tumor was found in a location where aortic bodies have not normally been observed, suggesting the possibility that it arose from an aortic body in an ectopic location, or that aortic bodies normally occur in locations other than those previously described.

Review of Literature

The carotid and aortic bodies are now considered to represent elements of a chemoreceptor system, but opposing views on classification and terminology are as yet unresolved, so that a brief review is presented.

The normal carotid body was first described in 1743 by von Haller,* who considered it to be an isolated ganglion of the cervical sympathetic trunk. Marchand † first described a tumor arising from this structure in 1891, and since that time several hundred have been recorded. In 1902 Wiesel ‡ reported a structure that is now recognized as an aortic body, situated near the origin of the left coronary artery. Similar collections of tissue were subsequently described between the aortic arch and the pulmonary artery, between the subclavian and the common carotid artery on the right side, and above the arch of the aorta internal to the left subclavian artery on that side of the body.[5] Aortic bodies in other locations have not been described, but Boyd [6] noted the difficulties of embryologic study of these organs, and their tendency to migrate away from their usual locations.

Neoplasms of aortic arch bodies were first recorded in dogs in 1943, when Bloom [7] reported 2 cases found among 500 necropsies, the tumors occurring at the base of the heart. Tumors of the base of the heart in animals, regardless of histologic type, are commonly referred to as "heart-base tumors" by veterinarians. Under this designation Nilsson,[8] in 1955, reported 40 heart-base tumors in dogs, of which 33 were considered to be aortic body tumors. In all reported cases in dogs metastases were absent. The only human aortic body tumors previously reported were two by Lattes,[9] in 1950. One was found in the upper mediastinum in the vicinity of the aortic arch and the innominate artery; the other, in the adventitia of the aortic arch near the obliterated ductus arteriosus. In neither instance were there metastases, but in the latter case two other independent carotid-body-like tumors were found, one on the vagus nerve at its exit from

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* References 1 and 2.

† References 3 and 4.

‡ Cited by Hollinshead [11] and Boyd.[6]

the skull and the other at the usual location for a carotid body tumor, at the bifurcation of the common carotid artery.

Terminology

Lattes advocated the term "nonchromaffin paraganglioma" for five groups of tumors arising from histologically similar organs: the carotid body, the glomus jugulare, the paraganglion intravagale, the paraganglion ciliare (described only in monkeys), and the aortic arch bodies. The background of this controversial term may be briefly outlined as follows: In 1900 Kohn[10] reported his investigations into the morphology and embryology of the carotid body, chiefly using pigs. He concluded that its cells showed a chromaffin-staining reaction, and that it arose in common with anlagen of the sympathetic ganglia. From this he gave it the designation of a paraganglion. Subsequent workers objected to this term, its use for carotid and aortic bodies implying (1) that the chief components of such organs arise from cells which also form the ganglion cells of the sympathetic nervous system; (2) that the cells of such organs have an endocrine function similar to those of the adrenal medulla, and (3) that such organs are intimately related to the peripheral nervous system. Evidence against using this terminology is the following[11]: (1) Only a small portion of these organs arises from the nervous system, and there is uncertainty on this point; (2) it is accepted that cells of the carotid and aortic bodies have no endocrine function; (3) these bodies have no relation to the peripheral nervous system; (4) true chromaffin tissue is related to motor fibers, while the carotid and aortic bodies receive chiefly sensory innervation from the glossopharyngeal and vagus nerves, and (5) the cells of the carotid and aortic bodies do not give a true chromaffin reaction.

Lattes recognized these arguments but proposed to extend the use of the term paraganglion to allow for these differences. LeCompte,[12] among others, has preferred the simple use of "tumor of the carotid body," etc., largely because of uncertainty concerning histogenesis of the main cells of these organs. From a review of the work of embryologists, anatomists, and physiologists, uncertainty concerning several aspects of this group of organs is obvious, and therefore the simple terminology of aortic body tumor is considered best suited to the neoplasm in this case report.

Report of a Case

The 79-year-old white man was brought to the accident floor of the Boston City Hospital vomiting copious amounts of bright-red blood and passing blood per rectum. According to members of his family, his vomiting had begun four hours earlier, following a meal. It had progressed to the point where on arrival at this hospital he was seen to retch up an estimated 500 cc. of blood. Physical examination, in addition to evidences of shock, revealed a large, firm liver 10 cm. below the right costal margin and bilateral cervical adenopathy. He died within minutes of arrival.

Past History.

He had been followed at another hospital for the previous eight years with diagnosis of Laennec's cirrhosis and syphilitic aortitis. Eight years before death he was first found to be anemic, with a hematocrit of 13% and a red blood count of 1,010,000 per cubic millimeter. His serology was positive; he gave a history of a primary syphilitic lesion at age 35, followed by treatment, commencing at age 57, consisting of courses of arsenicals and, later, of penicillin. A history of alcoholic intake from about the age of 20 was also obtained. Blood pressures were 120/60 and 110/62. The liver was not palpable. At the time of discharge the cause of the anemia had not been definitely established.

During an admission seven years before death the liver was palpated for the first time, 6 cm. below the right costal margin. The sclerae were slightly icteric; the total bilirubin of the serum was 5.6 mg. per 100 cc., and the direct bilirubin was 1.8 mg. per 100 cc. The serology was positive, but the anemia had improved on multiple hematinic drugs. Red blood counts were 3,210,000 and 2,750,000 per cubic millimeter. A gastrointestinal x-ray series showed a tortuous but otherwise negative esophagus. The last known

hospital admission was six years before death, at which time the serology was positive, and the red blood counts were 3,620,000, 2,830,000, and 2,750,000 per cubic millimeter. The liver was now palpable 10 cm. below the right costal margin and was firm. A slight questionable icteric tinge of the skin was noted. No neck masses were palpated.

Fasting blood sugar determinations were not performed during any of the above admissions, but urine examinations on each hospitalization had shown no glucosuria. Since his last hospital admission he had apparently been in fair health, living at home, until the terminating event of hematemesis.

Autopsy.

The cause of death was found to be ruptured esophageal varices with exsanguination. The stomach, duodenum, and small and large bowel contained an estimated 2000 cc. of blood. Hemochromatosis and fatty nutritional cirrhosis were found, with a hepatoma that had metastasized to portal, hilar, and cervical lymph nodes and to the lungs. There was a moderate-sized aneurysm of the ascending aorta, considered to have been syphilitic in origin. In addition, an unusual mass was found on the left subclavian artery. The mass was located immediately distal to the thyrocervical axis and completely encircled the left subclavian artery without significantly narrowing its lumen. No connection with the carotid artery or the vagus nerve was demonstrated. The tumor was oval, very firm, and had a fibrous encapsulation. On section it cut with a resisting, grating sensation. It measured 5 cm. in diameter and was composed of solid pink, meaty tissue, with numerous small vascular spaces traversing it and with a central linear red area of degeneration (Fig. 1). The gross impression was that it resembled a carotid body tumor, and, because of its unusual location, the carotid artery on that side of the neck was removed for examination. The cervical lymph nodes, including those about the carotid arteries, were involved by tumor, the appearance of which on section was of white soft friable tissue, easily distinguished from the mass on the subclavian artery, both grossly and microscopically.

Microscopic Examination: The tumor mass on the subclavian artery was composed of highly cellular tissue, chiefly large oval "epithelioid" cells with marked variation in size and shape. The nuclei were vesicular, with prominent variation in size and with pyknosis. Eosinophilic cytoplasm was prominent (Fig. 2); in areas, large cells formed a palisading about blood vessels. Rare giant cells were present; no mitotic figures were seen. Many blood vessels and sinusoidal spaces were present, with a hyaline degeneration in some perivascular spaces. The tumor tissue was enclosed by a fibrous capsule, which extended in abundant quantities into the tumor tissue itself. A brown granular pigment was prominent in tumor tissue, lying free; in rare areas capillaries about tumor masses gave the false impression of tumor cells within blood vessels. The *Zellballen*, or nests of uniform epithelioid cells which comprise the typical carotid body tumor, were not apparent with routine stains.

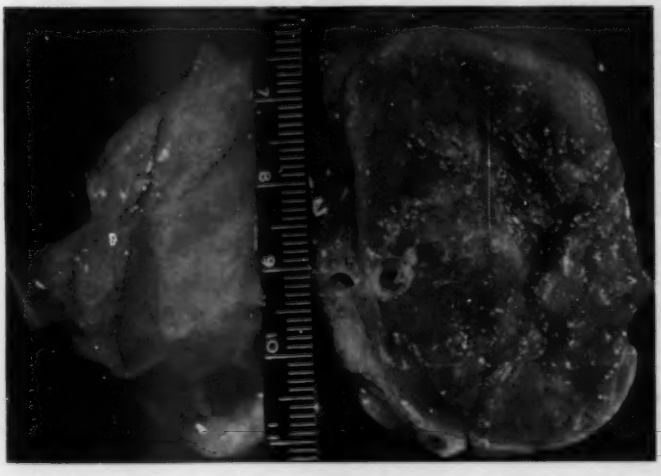

Fig. 1.—Close-up of mass on left subclavian artery in hemisection. Proximal portion of the subclavian artery and its thyrocervical axis is also shown.

With reticulum stains, however, a vascular fibrous stroma separated groups of cells (Fig. 3). There was no spread of this type of tumor to lymph nodes or other organs.

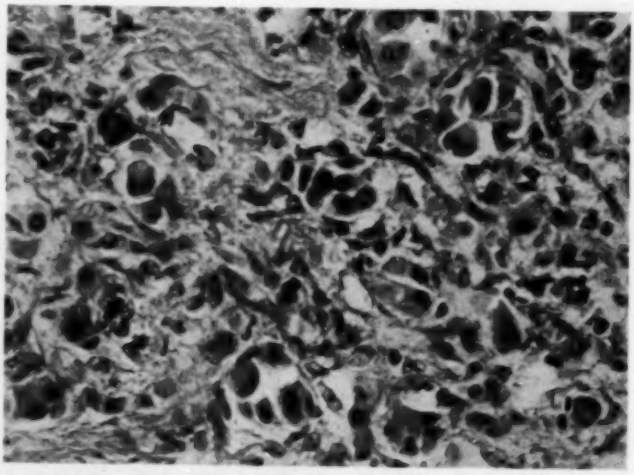

Fig. 2.—Photomicrograph of mass on left subclavian artery. Note large "epithelioid" cells with abundant cytoplasm and nuclear variation. Hematoxylin and eosin stain; reduced to 2/3 of mag. × 400.

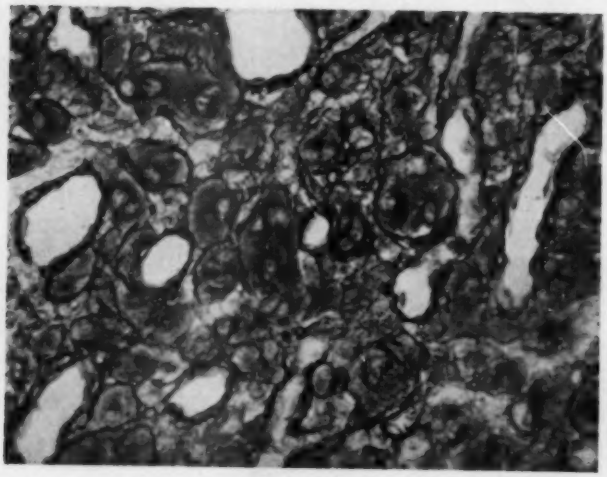

Fig. 3.—Photomicrograph of mass on left subclavian artery, stained for reticulum to illustrate that nests of cells are separated by reticulum fibers. Foot's modification of Bielschowsky silver stain for reticulum; reduced to 2/3 of mag. × 850.

Comment

The uncommon occurrence of this group of tumors is evidenced by the fact that in 124,442 consecutive surgical specimens at this Institute, 1900-1955 inclusive, 7 cases of carotid body tumor were found, and histologic sections were available for review. No instances of carotid or aortic body tumors had occurred previously in autopsy material, consisting of 27,026 autopsies, 1895-1955 inclusive, this case being the first such instance. None of the other types of tumors of this group had occurred in surgical or autopsy material.

Bloom and Nilsson, in their reports of aortic body tumors in dogs, illustrate histologic features that are different from typical carotid body tumors. The differences are chiefly the absence of the usual *Zellballen* formations with ordinary stains and the presence of what is referred to as peri-

theliomatous cuffing of tumor cells about blood vessels. These features appear to have been present in one of Lattes' human cases and occurred in the case here reported. These findings may indicate that aortic body and carotid body tumors have different typical histologic arrangements of cells, although a final evaluation must await the study of a large number of aortic body tumors. The typical pattern in carotid body tumors appears to be more definite nests of cells, while the pattern of aortic body tumors is one of loose sheets of cells containing areas with peritheliomatous formations. The reticulum pattern in the two types of tumor is the same and at the present time presents a helpful diagnostic staining characteristic.

Summary and Conclusions

A carotid-body-like tumor arising on the left subclavian artery in a man with hemochromatosis and hepatoma is described. It is considered to represent an aortic body tumor; a review of the literature indicates that this is the third case of a human aortic body tumor to be reported.

Dr. G. Kenneth Mallory furnished guidance in the preparation of this paper, and Dr. Philip LeCompte reviewed the histologic preparations of the aortic body tumor. Technical assistance was given by Miss Frances Murphy; photographs were by Mr. Leo Goodman.

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Biological Studies of Dihydrocholesterol

Production of Biliary Concrements and Inflammatory Lesions of the Biliary Tract in Rabbits

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ERWIN H. MOSBACH, Ph.D., New York

Introduction

In the course of studies of sterol metabolism in the rabbit [2] it was observed that animals receiving dietary supplements of dihydrocholesterol (DHC) consistently developed biliary concrements and inflammatory lesions of the biliary tract. It is the purpose of this report to define the conditions under which these pathological changes may be produced and to describe the nature of the lesions.

Methods

Male chinchilla-type rabbits, weighing 1.8 to 4.2 kg., were supplied by J. C. Landis, Hagerstown, Md. The animals were maintained in individual cages and received water ad libitum at all times. The basic diet was 100 gm. of Purina Rabbit Chow pellets. This quantity of food was usually consumed by the animals during a 24-hour period. The sterol-treated food was prepared as follows: The correct weight of sterol, DHC, cholesterol, or β-sitosterol, was dissolved in absolute ethyl ether, and this solution was poured over a weighed amount of the food pellets; the ether was then allowed to evaporate. The quantities of food and sterol were adjusted to yield the desired final sterol concentration (0.25%, 0.50%, or 1%). In many of the experiments olein (triolein) in the form of olive oil U.S.P. was also added to the diet in sufficient concentration to obtain a final concentration of 12% olive oil.

The experimental animals were killed at the end of 1 to 13 weeks, as indicated in the accompanying Table. The rabbits were killed by air embolism, and complete autopsies were performed. Sections of kidneys, liver, spleen, and aorta were fixed in formalin and Zenker's solution. Frozen sections, as well as paraffin-embedded sections, of these organs were examined. Special stains were employed as indicated.

Results

In the Table are listed the number of animals on each of the diets, the kinds of diet employed, and the duration of the feeding periods. The severity of the gross biliary lesions is indicated as follows: 0, no biliary lesion; ±, cloudy bile with a few minute concrements; ++, large concrements in the gall bladder but not within the intra- or extrahepatic ducts; and +++, large concrements, within both the gall bladder and the large intra- and extrahepatic ducts.

The Table shows that none of the 18 rabbits in the control groups developed biliary lesions, whereas in all animals fed DHC for one week or longer gross changes developed in their gall bladders. Two animals on the 1% DHC regimens (F and G) and one rabbit on control Diet A, had rudimentary gall bladders. In both DHC-fed animals, however, lesions of the biliary tree were present.

In all rabbits fed DHC for three to six weeks the gall bladders were filled with small concretions about 1 mm. in diameter, which frequently had coalesced to form a solid cast within the fundus (Fig. 1). The concretions were basically white but had a bluish-green appearance, due to the presence of a small quantity of bile pigment. When

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A preliminary report of this work has been presented.[1]

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From the Columbia University Research Division, Goldwater Memorial Hospital, Welfare Island, N. Y., and the Departments of Biochemistry, Medicine, and Pathology, Columbia University College of Physicians and Surgeons.

Effect of Various Diets upon Biliary Tract of Rabbits

	Regimen	Duration of Regimen, Wk.	No. of Animals	Gross Lesions*			
				Absent	+	++	+++
A.	Stock	3	7	7			
B	Stock + 12% olive oil	1 to 6	4	4			
C	Stock + 1% cholesterol	6 to 12	3	3			
D	Stock + 1% cholesterol + 12% olive oil	3	2	2			
E	Stock + 1% β-sitosterol	4	2	2			
	Total		18				
F	Stock + 1% DHC	3	7			2	5
G	Stock + 1% DHC + 12% olive oil.	1	1		1		
		2	1		1		
H	Stock + 0.5% DHC + 12% olive oil	3	2			1	1
		1	1		1		
		2	1			1	
		6	6			6	
I	Stock + 0.25% DHC + 12% olive oil	1	1		1		
		2	1		1		
		6	3			2	1
	Total		24				

* Number of animals having a given degree of gross lesions: +: inflammatory reaction of biliary tract and cloudy bile; ++: inflammatory reaction of biliary tract and concrements in gall bladder; +++: inflammatory reaction of biliary tract and concrements in gall bladder and bile ducts.

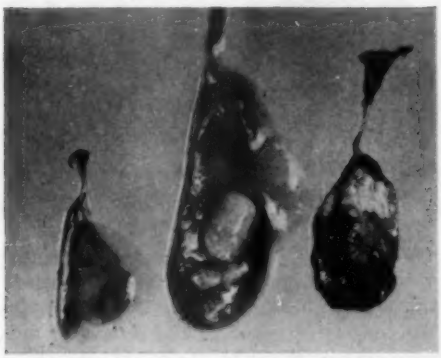

Fig. 1.—Gross lesions of the gall bladders of rabbits fed 1% DHC plus 12% olive oil for six weeks. The gall bladder of one of the control animals appears at the left. The other two gall bladders show stones in the fundus and in the common duct. The gall bladder at the right contained a single large cast within the fundus.

the concrements had coalesced to form a single stone, the latter was usually a dirty greenish-brown, due to the inspissated bile.

The walls of the gall bladders of all DHC-fed animals appeared to be slightly edematous. Portions of the concretions and bile clung tenaciously to the mucosal surface; however, after they had been removed by washing, the mucosa was found to be smooth and regular. When concretions were present in the large intrahepatic ducts, they caused sufficient distention to make the ducts visible in the periphery of the liver.

Microscopic examination of the gall bladder of the animals receiving stock diet con-

Fig. 2.—Normal gall bladder of a control rabbit, for contrast with Figures 3 and 4. Hematoxylin-eosin stain; × 92.

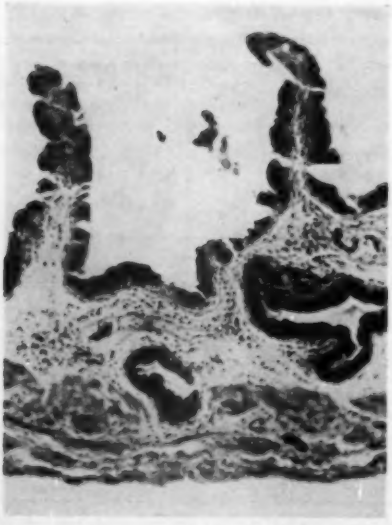

Fig. 3.—Lesions of the gall bladder in a rabbit fed 1% DHC plus 12% olive oil for eight days. The lamina propria is edematous. There are lymphocytes and histiocytes infiltrating this layer, which tend to have a perivascular arrangement. Hematoxylin-eosin stain; × 92.

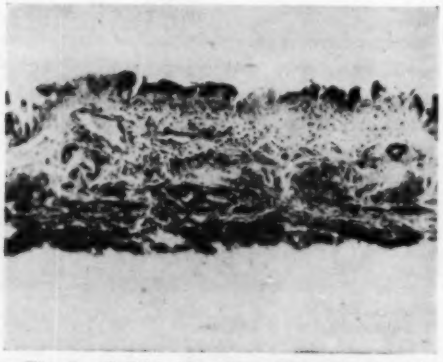

Fig. 4.—Gall bladder of a rabbit fed 1% DHC plus 12% olive oil for three weeks. Note flattening of the mucosal folds, thickening of the lamina propria, and infiltration of this layer by lymphocytes and histiocytes. The serosa appears thickened by fibrous tissue. Hematoxylin-eosin stain; reduced to 80% of mag. × 92.

taining 1% DHC for a period of three weeks (Diet F) revealed slight thickening of the mucosal folds due to edema; the folds were occasionally adherent (Figs. 2 and 3). There were numbers of lymphocytes and wandering cells, especially in the lamina propria, where the edema was most extensive. The cellular infiltration tended to have a perivascular arrangement. The muscularis was not altered except by edema. The serosa was thickened by edema and often by fibrous tissue. The gall bladders of the DHC-fed rabbits were slightly more adherent to the liver than normal. The lesions in the large ducts were similar to those found in the gall bladder, but were generally severer. Some of Group F showed a flattening of the mucosal folds of the gall bladder, possibly due to the distention or the pressure exerted by the concrements (Fig. 4). However, there was no significant increase in cellular infiltration or proliferation of fibrous tissue. After the animals had received 1% DHC plus olive oil for six weeks (Diet G), there was minimal proliferation of fibrous tissue in the lamina propria.

The addition of 12% olive oil to the DHC-containing diet (Groups G, H, and I) did not produce significant changes in the severity of the inflammatory lesions of the biliary tract. However, fat was demonstrated in the lamina propria of the gall bladder and the intra- and extrahepatic bile ducts, as well as in the parenchymal cells of the liver (Fig. 5). The sudanophilic material in the animals fed DHC plus stock diet without olive oil was limited to the serosa of the gall bladder, the Kupffer cells and the connective tissue of the periportal radicles about the bile ducts of the liver (Fig. 6).

The gall bladders of the animals receiving the stock diet plus 12% olive oil (Diet B) showed no inflammatory response, but the bile, although clear and translucent, was dark greenish-brown and slightly viscid, in contrast to normal rabbit bile seen in the animals of Group A. Animals receiving 0.25%, 0.50%, or 1.0% DHC together with added oil (Diets G, H, F) all developed inflammatory lesions of comparable severity to those induced by Diet F (1% DHC without added oil). Moreover, there appeared to be little change in the severity of the tissue reaction in the biliary tract with

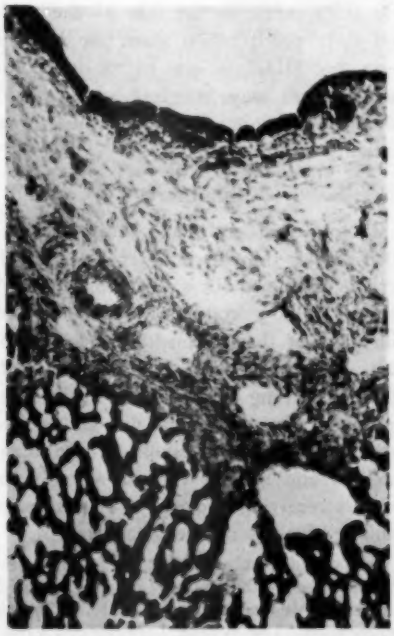

Fig. 5.—Lesion in an intrahepatic bile duct in a rabbit fed 1% DHC with 12% olive oil. The lesion in the duct is similar to that of the gall bladder. Lipid is present in the lamina propria, as well as in the serosa of the duct, and is abundant within the parenchymal cells of the liver. This is indicated by the density of the liver cells. Oil red O; × 90.

Fig. 6.—For contrast with Figure 5. Intrahepatic duct of a rabbit fed 1% DHC for three weeks. Note the edema and cellular infiltration throughout the wall. Lipid is seen in the outer serosa adjacent to the parenchyma. It is also present in the Kupffer cells, but cannot be seen at this magnification. Oil red O; × 90.

prolonged feeding of the experimental diet. The inflammatory reaction observed after one week of DHC feeding was essentially unchanged after six weeks of the DHC regimen, except for a slight thickening of the lamina propria by fibrous tissue. This finding is in contrast to the appearance of the contents of the biliary tract; the number and size of the concrements increased with the prolongation of the feeding period and with an increase of the DHC content of the diet from 0.25 to 1.0%.

The kidneys and aortas of the rabbits showed no alterations attributable to the DHC regimens. The spleen showed occasional lipid-laden macrophages, which were observed in all animals receiving DHC, with or without added olein. Spontaneous medial lesions of the aorta were present in the majority of the rabbits, but no intimal lipid infiltration was observed in these experiments, except in cholesterol-fed control animals (Groups C and D).

None of the DHC-fed rabbits used in this study was observed to appear ill or to develop jaundice. This was true even for those animals which had received DHC for six weeks and had severely obstructed bile ducts. It has been shown, however, that even rabbits with ligated bile ducts develop only transitory jaundice, which may escape the casual observer.*

Comment

The consistent production of gallstones in rabbits by dietary means has not been reported previously, although Cook, Kliman, and Fieser [3] found stones and murky bile

* Wilens, S. L.: Personal communication to the authors.

in two of four rabbits receiving DHC for 23 days. In the present study it was shown that the administration of 250 mg. of DHC per day for a period of only one week produced changes in the appearance of the bile and caused an inflammatory reaction throughout the billiary tree. It seems likely that the inflammatory reaction of the bile passages was caused by dietary DHC, and that this sterol is therefore responsible, either directly or via one of its metabolites, for the production of the biliary calculi. Liaw [4] reported the production of gallstones in 62% of rabbits suffering from chronic damage of the biliary system, caused by injecting a mixture of the sodium salt of a free bile acid and a fatty acid into the gall bladder. In addition, it has been shown that cholesterol-fed guinea pigs which developed gallstones on diets containing excessive amounts of riboflavin exhibited an "irritation of the gall bladder and biliary passages and some impaction of ducts."[5] Dam has shown that gallstones of high cholesterol content are produced in hamsters on cholesterol-free diets but was unable to correlate his findings with changes of the cholesterol metabolism of the experimental animals or with a specific agent contained in the diet.†

In the present investigation, it was demonstrated that DHC, in contrast to cholesterol or β-sitosterol, has a specific irritating effect upon the biliary tree of the rabbit. It seems likely that the inflammatory response either precedes the formation of the biliary concrements or occurs simultaneously with it.

Although the rabbit can absorb large amounts of DHC from the gastrointestinal tract and store this sterol in its tissues,‡ the biliary concretions contained less than 0.02% of digitonin-precipitable sterols.§ The gallstones consisted largely of deoxycholic and glycocholic acids, with the former predominating.§ These conjugated bile acids occur in the bile of the rabbit, and their precipitation from the bile suggests that DHC, or one of its metabolites, may in some way interfere with the solubility relationship of normal biliary constituents.

† References 6-10.

‡ References 2, 3, 11.

§ Mosbach, E. H., and Bevans, M., to be published.

Summary

Rabbits receiving diets containing 250 to 1000 mg. of dihydrocholesterol per day for periods of two to six weeks consistently developed biliary concrements and a mild phlegmon of the gall bladder and large bile ducts. The addition of 12% olive oil to the DHC-containing diets increased the concentration of liver lipid but did not increase the severity of the lesions of the biliary tract. None of the control animals, nor animals receiving cholesterol or β-sitosterol exhibited lesions of the biliary tract.

The dihydrocholesterol used in this study was supplied by the courtesy of The Squibb Institute for Medical Research, and the Schering Corporation. The Wilson Laboratories gave us some of the cholesterol.

J. Bruder, F. Drimmer, E. Halpern, and R. Kaplan gave technical assistance.

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Natural History of Degenerative Joint Disease in Small Laboratory Animals

I. Pathologic Anatomy of Degenerative Joint Disease in Mice

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Degenerative joint disease (osteoarthritis) is an important problem of public health. The lesions are present to a certain degree in virtually all persons entering the middle years and become progressively severer as the decades pass by.* Aside from incapacitating many elderly persons, there is reason to believe that osteoarthritis accounts for more absences than any other type of joint disease in industry † and in the armed forces.[5]

The abrasion that is characteristic of the lesions of osteoarthritis and their localization in individual joints, while others are spared, have stigmatized mechanical destruction of the articular cartilage as an essential pathological process in the development of degenerative joint disease. It is generally believed that osteoarthritis is an inexorable erosion of old age. The paucity of research into the possibility that the development of degenerative joint disease may be modified or hindered must be attributed in large part to this conviction.

It has long been known that trauma and disadvantageous posture may induce osteoarthritis even in young and previously healthy articular cartilage. This is often referred to as a secondary type of osteoarthritis, in distinction to the primary type, to which no specific traumatic or postural etiology can be assigned. It is recognized that the division between the two cannot always be made sharply. Insofar as a secondary type exists, degeneration of the articular cartilage, whether due to aging or to metabolic disturbance, is not a prerequisite for the development of osteoarthritis. In this sense, Collins has written [6]:

> The inciting conditions of the disease must be sought separately for each diseased joint, for the general or systemic influences in osteoarthritis . . . are either rare or seemingly unimportant.

Nevertheless, osteoarthritis is by and large a disorder of the later decades of life, when a number of regressive changes are known to occur in cartilage.[7] That involution of the articular cartilage may affect its vulnerability to mechanical erosion appears reasonable. The dependence of the integrity of the cartilage on its own metabolic activities offers one main avenue of approach to the possible control of degenerative joint disease. There are, in fact, a number of evidences from the pathology of human osteoarthritis that systemic factors may play an important contributory role.

1. There is an increasing appreciation of the significance of nutrition of the articular cartilage (albeit local), as opposed to the mechanical trauma of weight bearing, in the development of articular degeneration.‡

2. There is an increasing appreciation of the fact that weight bearing cannot be the principal factor implicated in certain pat-

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National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Department of Health, Education, and Welfare.

* References 1: (*a*) p. 42; (*b*) p. 44; (*c*) p. 65. Reference 2: (*a*) p. 81; (*b*) p. 102.

† Reference 3. Brown, R.; Lingg, C., and Mainland, D., cited by Bunim.[4]

‡ References 6, 8.

terms of generalized osteoarthritis.§

3. There is at least one outstanding metabolic disorder that causes articular cartilage to deteriorate and then undergo osteoarthritic change—ochronosis.[11]

Osteoarthritis occurs widely among bulky mammals.‖ It was not recognized in small laboratory rodents until recent years. In a survey of wild mammals, Fox[14] studied joints from 173 rodents. In none of these was arthritis apparent. This investigator cited a communication from Dr. A. E. Hamerton, of the London Zoological Society, to the effect that he had had a similar experience in a study of arthritis over a period of 15 years. A significant advance in this direction came with the observations by Silberberg and Silberberg[15] that osteoarthritis of microscopic proportion does indeed occur in mice under certain conditions. In a series of provocative reports, these investigators have presented evidence that development of degenerative joint disease is influenced profoundly by a variety of nutritional and hormonal factors.[16]

An extensive anatomical examination of the joints of small laboratory rodents has been made as a basis for an experimental study of the pathogenesis of degenerative arthritis. The present report deals with several aspects of this study. Its goals have been several:

(1) to determine whether the degenerative joint disease of these animals is comparable pathologically to that of man;

(2) to determine which animals and which joints are most suitable for study;

(3) to determine whether degenerative joint disease is an expression of skeletal aging as measured by other modalities;

(4) to determine what factors may profitably be explored in future investigations;

(5) to provide a useful anatomical guide in a field that will increasingly claim the attention of investigators concerned with diseases of aging.

§ References 9, 10.

‖ References 12-14.

Methods and Materials

Joints of 670 mice between 12 and 22 months of age have been examined histologically. The mice comprised 18 strains from the Inbred Colony of the National Institutes of Health. Details concerning the strains, age, sex, and degree of inbreeding are presented elsewhere.[20] After being retired from the breeding area, they were maintained concurrently in a single room under identical conditions. They were housed in plastic cages in groups of four to seven. The floor of the cages was covered with wood shavings. The temperature of the room was maintained at 78-82 F, although there was some occasional fluctuation a few degrees above and below this range. The diet consisted of Purina Fox Chow and water ad libitum, supplemented twice a week by a ration of kale. Some of the animals were killed; others died spontaneously. Autolytic changes in the latter were rarely sufficiently severe to preclude histologic diagnosis.

In addition to the microscopic study, macerated skeletons of 59 other mice from this colony, and 38 others made available by Dr. Michael Potter were examined under a dissecting microscope. There were prepared by the papain technique of Searle.[28]

The bony carcass was fixed in neutral 10% formalin. The bones were decalcified with 5% formic acid in water. The acid was changed twice daily until the decanted fluid no longer reacted with saturated oxalate to form a precipitate. The tissues were embedded in Tissuemat (Fisher). Sections were made at a thickness of 8μ. The ribbons were floated onto a water bath in which a pinch of gelatin (Difco certified, Difco Laboratories, Inc., Detroit) had been dissolved. In each instance, sections were made from both knees. At least three, and usually four, sagittal levels were studied in each knee. The sections were designed to pass through the medial condyles and through the level of the patella. In several strains it was discovered that there was a predilection for the lateral condyles to be involved. In these groups additional sections were made through the lateral condyle as well. The sections were stained routinely with Delafield's hematoxylin and eosin method.

A survey of the distribution of degenerative changes in the various diarthrodial joints was carried out in 12 mice. Serial sections of 21 pairs of joints were made in each of these. The structures studied are listed in the accompanying Table.

A number of special histochemical procedures were carried out on normal and degenerated cartilage. The methods employed were those

*Distribution of Osteoarthritis in Twelve Mice**

Joint	C3HfB/HeN 20 Mo. Female		DBA/2JN 13 Mo. Male		STR/1N 21 Mo. Female		STR/1N 21 Mo. Female		STR/1N 21 Mo. Male		STR/1N 21 Mo. Female		C3HfB/HeN 19 Mo. Female		A/HeN 20 Mo. Male		DBA/2JN 21 Mo. Female		DBA/2JN 21 Mo. Male		STR/1N 20 Mo. Male		BRSUNT/N 21 Mo. Female	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Temporomandibular	±	0	0	0	0	–	0	0	0	0	0	0	0	0	±		0	0	0	0	1+	0	0	0
Scapulohumeral	0	0	0	0	0	0	0	0	2-3+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Humeroulnar	0	0	0	1+	1-2+	1+	2+	0	0	1+	1-2+	0	0	0	0	0	0	0	±	0	0	±	0	0
Radiocarpal	0	0	0	0	0	0	1+	±	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Carpal	0	0	0	0	0	0	1+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpometacarpal	0	0	0	±	0	0	0	2+	0	0	1+	1+	0	0	0	0	0	0	0	±	0	0	0	0
Metacarpophalangeal III	0	0	0	0	0	0	0	0	0	1+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximal interphalangeal (forepaw) III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal interphalangeal (forepaw) III	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0	0	0	0	0
Coxofemoral	0	0	0	±	±	±	0	0	0	0	0	0	0	0	0	0	±	±	0	0	0	0	0	0
Knee	3+	0	2+	0	0	1+	3+	3-4+	4+	4+	3+	3-4+	0	0	0	1+	3+	2-3+	2+	3-4+	4+	3-4+	4+	3+
Tibiotalar and talocalcaneal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2+	1-2+	0	0	0	0	1+	0	0	0
Tarsal	0	0	1-2+	2+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0
Tarsometatarsal III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±	±	0	0	1+	2-3+	0	0	0	0
Metatarsophalangeal III	0	0	0	0	0	0	0	1-2+	0	0	±	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximal interphalangeal (hindpaw) III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal interphalangeal (hindpaw) III	0	0	–	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sacroiliac	±	±	0	–	1+	1+	1+	1+	1+	1+	0	0	1+	1+	±	±	±	±	1+	±	±	±	±	±
Apophyseal, thoracic vertebrae	±	–	0	–	0	±	2+	2+	0	0	0	0	0	0	0	0	±	0	0	0	±		0	0
Capital c.v.	±	–	0	–	0	–	2+	2+	1-2+	1-2+	0	0	±	±	0	0	±	0	0	0	0	0	0	0
Tubercular c.v.†	0	–	0	–	–	–	±	±	±	±	1+	–	0	0	±	0	0	0	0	0	0	0	0	0

* Severity of lesions is graded 0 to 4+, as indicated in text.
† c.v. indicates costovertebral.

described in Lillie's textbook.[23] They included staining with azure A-eosinate at pH 3.2; Rinehart's modification of Hale's stain; Wilder's and Van Gieson's; allochrome; dilute aqueous toluidine blue; alkaline phosphatase following fixation in chilled 65% ethyl alcohol; acid phosphatase and lipase following fixation in chilled acetone; periodic acid-Schiff technique, with and without diastase; frozen sections stained with oil red O, and examination under the polarizing microscope.

Histologic Changes in Osteoarthritis of Mice

The earliest lesions usually take the form of a localized disintegration of articular cartilage (Fig. 1*a*). In these regions, many hematoxylin-eosin preparations. Not infreis redder than in the viable tissue in the hematoxylin-eosin preparations. Not infrequently, side by side with these, there are a number of somewhat enlarged chondrocytes and lacunae that are surrounded by blue-stained haloes. Irregularly shaped fissures appear in the altered matrix substance. The fissures usually extend to the zone of the calcified cartilage. A pattern of radical fibrillation is not present as uniformly as in the human lesion. When it

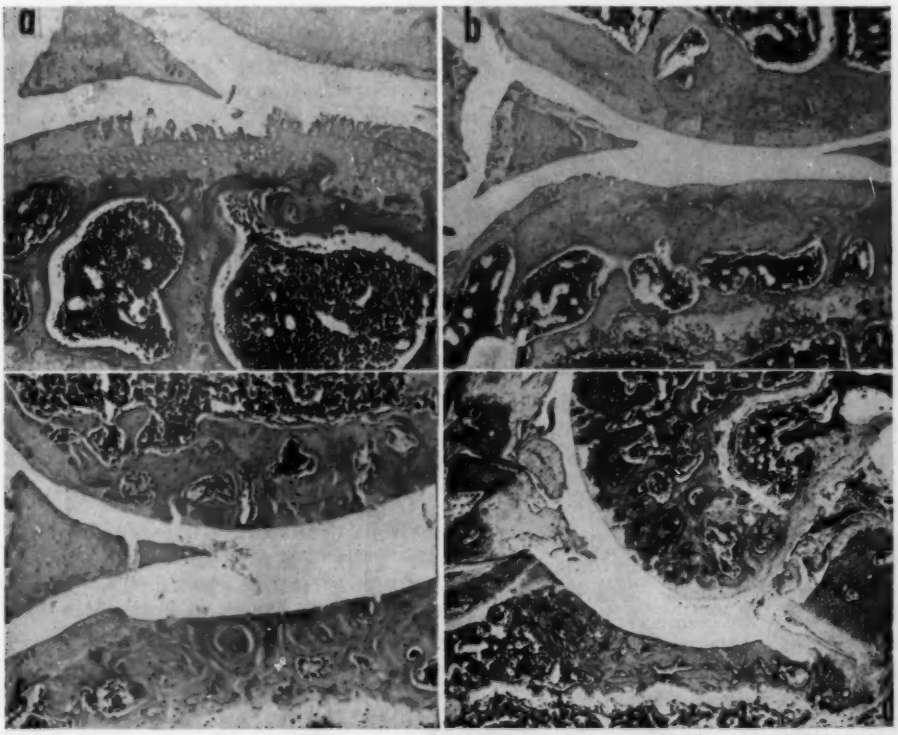

Fig. 1.—Osteoarthritis of the knee of mice, graded according to criteria described in test.

(*a*) 1+ osteoarthritis. A small area of fibrillation and disruption of articular cartilage is present in the head of the tibia. Hematoxylin and eosin; reduced to 73% of mag. × 105.

(*b*) 2+ osteoarthritis. Much of the condyle of the femur and head of the tibia is denuded of articular cartilage. The erosion does not extend beyond the zone of calcified cartilage. Proliferative changes are seen in the chondrocytes in the residual articular cartilage and in the meniscus. Hematoxylin and eosin; reduced to 73% of mag. × 105.

(*c*) 3+ osteoarthritis. Erosion has progressed into the epiphysis of the head of the tibia. There is an increase of bone in the epiphyses adjacent to the articular defects. Hematoxylin and eosin; reduced to 73% of mag. × 105.

(*d*) 4+ osteoarthritis. A large concave defect is present in the epiphysis of the head of the tibia. Hematoxylin and eosin, reduced to 73% of mag. × 45.

does occur, it does so most frequently in the region of attachment of the intra-articular ligaments.

Following the initial disintegration, a horizontal cleft appears between the articular cartilage and the zone of calcification. Necrotic fragments of articular cartilage are detached and often can be identified as displaced sequestra in the synovial membrane.

In more advanced lesions, erosion progresses through the calcified zone into the epiphyses. Ultimately, large subchondral defects appear. In the case of the knee, excavation takes place in the head of the tibia. Sclerosis of the bone adjacent to the defects occurs in a manner quite comparable to the eburnation of the human lesion (Fig. 1*d*).

Changes in the soft tissues of the joints are more conspicuous than in the human disease. Posterior displacement of the capsule of the joint in the region of the medial condyle of the tibia is accompanied variously by fibroblastic, cartilaginous, and ossific proliferation at the junction of the capsule with periosteum. This frequently results in formation of exostoses. Intra-articular osteophytes, however, are rare. Periarticular fibrosis, mucoid degeneration, and cyst formation develop primarily in the popliteal space, in the vicinity of the capsular-periosteal cellular activity. In portions of its popliteal aspect, the fibrous joint capsule is normally thin or discontinuous. Compression and distortion of minute synovial recesses and the adjacent popliteal adipose tissue are seen early in the development of the popliteal changes. It appears likely that the popliteal tissues are subjected to trophic disturbances that arise mechanically from the damage to the articular cartilage. An analogous mechanism is generally presumed to operate in the formation of marginal osteophytes.¶ Proliferation of synovial lining cells and fibroblasts usually accompanies the popliteal changes. It also frequently, at least in part, is a reaction to articular detritus that has become sequestrated in the synovial membrane. A similar pattern of detritic synovitis has been reproduced experimentally in the rabbit.[25] Columnar enlargement of the lining cells occurs to a mild degree in some animals in association with degeneration of articular cartilage in the apparent absence of articular erosion. The cruciate ligaments frequently undergo changes analogous to those in the articular cartilage. Frequently they appear thickened. In large part the thickening may be attributed to collapse of the joint. Fraying of the ligaments is common, but total disruption is rare. The menisci also participate in the destructive process. In most instances of moderate severity their internal portions are detached. Focal proliferation of articular chondrocytes may accompany the proliferative reaction of the other joint surfaces.

On the basis of this interpretation, the following scheme for grading the severity of the process of osteoarthritis has been employed:

1+: A small defect, no larger than a quarter or so of the length of the articular surface in a single section, is present and confined to the noncalcified articular cartilage (Fig. 1*a*).

2+: A large defect in the articular cartilage is present in one or more surfaces but does not penetrate through the zone of calcified cartilage (Fig. 1*b*).

3+: Erosion extends into the subchondral plate of bone but does not lead to a large deformity (Fig. 1*c*).

4+: Erosion becomes so extensive that major deformity of the joint results (Fig. 1*d*).

A special pattern of separation of articular cartilage has been observed in high incidence in the lateral condyle of several strains. This is characterized by an isolated cleft of dissection along the zone of calcification. The cleft is usually present bilaterally. It often extends on one aspect to a minute crevice in the articular surface, where cellular degeneration of the type already described is present. Nevertheless, in the earliest changes most of the cartilage in the region of the cleft is viable. This cleft is believed not to be an artifact, since (1) when is is possible to trace the limits of the lesion serially, a minute crevice of

¶ Bennett, Waine, and Bauer,[1] p. 65.

the sort already described can usually be demonstrated at the surface on one end, (2) it is a bilateral lesion; (3) it occurs only in closely related genetic strains, (4) it is in these strains that osteoarthritis develops in the lateral, rather than in the medial, condyles, the clefts also being confined to the lateral condyles. A similar type of separation was recognized in the human by Pommer,[24] who regarded it as a histologic stigma of traumatic arthritis.

When such a cleft is associated with a demonstrated crevice in the articular surface, or when there is a focus of disintegration or fibrillation of articular cartilage without a defect in its continuity, a ± grade of osteoarthritis is recorded.

Distribution of Degenerative Joint Disease in Mice

This study was carried out to determine (1) whether the osteoarthritis of mice has a predilection to occur in certain localities where mechanical influences can be implicated as playing a critical pathogenetic role, or whether a generalized senescence of articular cartilage is involved; (2) what joints are most suitable for study, and (3) whether any correlation can be established between the occurrence of arthritis and generalized degeneration of nonarthritic articular and other hyaline cartilage.

It is seen in the Table that the knee joint is by far the area most severely affected. The lesions usually occur in symmetrical fashion on both sides of the body, but this is not always so. The medial condyles are affected to a much greater degree than the lateral in most strains studied. In the more advanced grades, excavation of the tibial epiphysis occurs, principally on the dorsal aspect. The femur may become subluxated in this region (Fig. 2). It has already been pointed out that it is here that soft-tissue changes and osteophyte formation develop most frequently.

The involvement of the knee may be related mechanically to the fact that the animal frequently supports itself on the hind extremities. In the bulky quadrupeds, the lesions are severest in the foreparts of the skeleton, and this corresponds, in a general way, to the posture of the animals with respect to weight bearing and receiving jolt shock.[14]

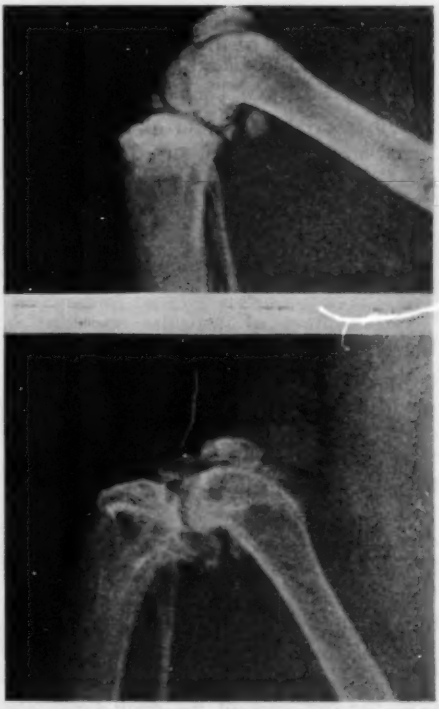

Fig 2.—Roentgenograms of knee of mice: above, normal; below, osteoarthritis. In the arthritic knee, the condyles of femur have become subluxated dorsal to the head of the tibia, and occupy a concave defect in the latter. A sclerotic rim of bone has formed at the margins of the tibial erosions. (Exposure factors: Dynamox "40" rotating anode tube: focal-spot size 1.5 mm.; 200 ma.; 32-35 kv. peak; 36-in. distance; 1/10 second; Eastman Kodak No-Screen film. The animals were anesthetized lightly.)

In several strains, different portions of the knee joint are affected. In the closely related C57L/HeN and C57BR/cdJN strains, the lateral condyle is involved in arthritis and in the separation clefts, whereas the medial condyle is spared. In the BRSUNT/N strain, and the related strains BALB/cAnN and BL/HeN, isolated erosions of the patella and intercondylar fossa of the femur appear commonly unlike other groups. It may be presumed that structural

differences among those strains are involved in the localization of the lesions in the knee. At the present time no postural or structural factor has been identified. Gross variation in the relative width of the bones is found among several of the strains. Measurements of this width and gross assessment of the contours of the macerated preparations have failed to yield a correlation with the localization or severity of the arthritis.

Degeneration of articular cartilage is found to occur in certain areas in the absence of osteoarthritis. As in the case of the arthritic disintegration, there is a segmental necrosis of chondrocytes, more intense red staining of the matrix in the hematoxylin-eosin preparation, and a scattering of chondrocytes that have a perilacunar collar of increased affinity for hematoxylin. These changes have been found to occur during the second year of life, in all animals examined, in the small joints of the extremities, the tibiotalar and radiocarpal articulations. In the small rodent, the cartilages of the small bones are one cell thick for the most part and have other structural peculiarities, e.g., scalloped borders.[26] To a much less extent, segmental changes occur in other joints too. It has not proved possible to correlate the degree of degeneration in the small articular cartilages with development of osteoarthritis in the knee. The same is true for the more extensively studied hip[21] and the universal amianthoid degeneration of the costal cartilages. The latter is like that described in the rat by Dawson and Spark.[27] A lack of correspondence between costal cartilage degeneration and osteoarthritis in man has been noted by other investigators.[28]

In the arthritic joint, the degenerative changes are often confined to the area of erosion. Elsewhere in the same joint the articular cartilage may appear normal.

Gross Changes in Osteoarthritis of Mice

No disturbance of gait has been recognized in the arthritic animals. The lesions develop at an age when the animals are relatively sedentary, whether they have arthritis or not. Gross changes are appreciated only in the more advanced lesions. The knee joint appears somewhat enlarged, and its capsule, more opaque than normal and thickened. At times, the patella appears to be displaced medially and the tibia rotated internally on the femur. It is of interest that experimental displacement of the patella in the rabbit results in the rapid development of osteoarthritis.[29]

Fig. 3.—Macerated knees of mice, dorsal aspect: left, osteoarthritis; right, normal. The eburnated surfaces of the eroded femoral condyles have glistening highlights, unlike the normal counterparts. The corresponding articular surface of the head of the tibia is excavated and has a number of irregular marginal bony excrescences.

In the macerated preparations, the eburnated surfaces appear glistening rather than dull (Fig. 3). Deep erosions and the osteophytes described previously are found in the head of the tibia. Other types of osteophytes and deformities occur at times. They are distinguished from these below. The potential usefulness of the papain technique in this type of research is limited by the fact that only Grade 3 and Grade 4 lesions can be recognized with it. Roentgenography of the joints, under conditions compatible with life of the tissues, has proved even less useful in our hands.

Histochemistry of Osteoarthritis in Mice

These studies have been limited. The outstanding finding is a diminution of metachromatic material of the matrix in the regions of the necrosis of cells, as seen with the azure A-eosinate stain. It is presumed to reflect a loss of chondroitin sulfate from the region. A diminution of the chondroitin sulfate content of the osteoarthritic articular cartilage of man, as compared with the collagen content, has been found by chemical analysis.[30] Some slight reduction in the intensity of the periodic acid-Schiff staining of the matrix takes place in advanced lesions. Blue-staining material seen in the lacunae and capsules of the chondrocytes of viable articular cartilage with Rinehart's stain disappears from the zones of necrosis. There is no stainable fat in the cells or matrix of the arthritic cartilage. This finding is a matter of some interest in view of the suggestion that fat may be used excessively by articular cartilage in mice receiving a high-fat diet.[17] Excessive fat is found at times in the chondrocytes and matrix in human osteoarthritic cartilage.[31]

Miscellaneous Changes in Joints of Mice to be Distinguished from Osteoarthritis

Small areas of amianthoid degeneration are found quite frequently in the labrum glenoidale of the hip. Minute defects in the labrum may appear in this region. Associated with the degeneration, there may occur some calcification and ossific metaplasia in the labrum. Grossly this may be recognized in the form of a minute osteophyte on the rim of the acetabulum. It is not associated with osteoarthritis of the hip in the form already described, such arthritis being seen in only 3 of 183 pairs of hips sectioned.

Minute excrescences of calcific material and bone may be found at times near the ends of the bones, and not be related to the joint surfaces or osteoarthritis. Some of these clearly are the sites of attachments of articular ligaments or tendons.[32] In other instances the origin of such exostoses is unknown.

Cystic degeneration of articular cartilage, of the type often found in the rat, is found with much less frequency in the mouse.

In the marrow of the epiphysis of the head of the tibia, circumscribed cysts lined by fibrous tissue or bone are common. At times these cysts contain a somewhat mucoid fluid; in others, they are filled with fibrous tissue of loose texture. Serial sections reveal that they are contiguous to the synovial recesses at the insertion of the articular ligaments into the bone. They are not confined to knees affected by osteoarthritis, though they are quite similar to the popliteal cysts described above in the arthritic mice and to the subchondral cysts that occur in human osteoarthritis. It appears likely that they are trophic changes, related to the intrusion of synovial fluid or the compression resulting from this, through the discontinuous areas in the cortex of bone where the ligaments are inserted. A similar mechanism has been suggested as operating in the formation of subchondral cysts in human osteoarthritis.[33] Epiphyseal deformity and arthritis secondary to necrosis of bone occur in infrequent instances.[34] Hematogenous infectious arthritis is rare.

Comment

The morbid anatomy of osteoarthritis in man may be summarized briefly to allow comparison with the murine lesions. Pathologists are generally in agreement that the earliest gross changes consist of small, localized areas of splitting of the articular cartilage along its fibrillary planes.# These are followed by denudation of the cartilage from the underlying zone of calcification and the subchondral plate of bone. Alteration of the contour of the articulating surfaces follows as the destruction of the cartilage becomes more extensive. Proliferation of new bone and cartilage occurs subjacent to the erosions and at the margins of the articular surface. These changes are generally regarded as reactive phenomena. It is quite characteristic of osteoarthritis

References 1 and 2.

that it involves only certain of the diarthrodial joints. Even within the joint involved, there is a predilection for certain portions to be affected while others are spared. This is true in the bulky mammals,[14] as well in as man.[15] The distribution of the lesions has generally been correlated with weight bearing and other mechanical influences on the articular surfaces. Indeed, this very localized distribution of the changes constitutes one of the main pillars of the widely held view that osteoarthritis is a traumatic or destructive lesion, rather than a degenerative one.

The sequence of histologic events in the inception of the lesion is not certain. Aside from a progressive diminution in the number of chondrocytes that appears to take place with aging,* three intimately associated components of the tissue reaction are found in the immediate vicinity of the fissures in the cartilages: (1) local proliferation of chondrocytes; (2) local necrosis of chondrocytes, and (3) alteration of the chromophilic properties of the intercellular matrix. Our experience is in accordance with that of Collins, that the matrix here is stained pinker than normal in the hematoxylin-eosin preparations.†

Degenerative joint disease in the mouse shares fundamental anatomical similarities with the lesion that occurs in man. It originates in focal areas of abrasion and degeneration of articular cartilage. Extensive detachment of articular cartilage ensues. Ultimately, erosion results in deformity of the contours of the articulating surfaces, and reactive sclerosis develops. As in man and the bulky mammals, the lesion is not a generalized one but is prone to occur in certain portions of certain joints. Involvement of the knee may be correlated with the postural characteristics of the mouse, since the animal frequently supports itself on its hindlegs. The disease occurs in older animals.

So far as the sequence of histologic events in the tissues is concerned, the findings are susceptible of several interpretations, as they have been in man. We are in agreement with the statement of Bennett and co-workers‡:

> By the methods so far applied, it has been impossible to determine whether a disorder of the chondrocytes precedes the structural alteration demonstrable in the matrix. A priori reasoning and differential staining of the pericellular matrix suggest that structural alteration is a product of cellular activity.

The individual histologic changes are quite alike in the human and in the murine lesions, and the minor differences can probably be interpreted as a result of the difference in the size of the structures involved and the rate at which the lesions evolve.

Although retrogressive changes, in the form of localized necrosis of chondrocytes and loss of metachromatic material from the matrix, are frequently present in articular cartilage of various joints not affected by osteoarthritis and in nonarticular hyaline cartilage, it has not been possible to demonstrate that these changes are correlated with the development of osteoarthritis in the same animals. These changes, along with hypertrophy and hyperplasia of articular chondrocytes, are a feature of the articular cartilage defects that can be induced experimentally by mechanical means.[35] For this reason, it is not possible to state to what extent the cellular changes in the cartilage affected by osteoarthritis represent a primary degeneration and to what extent they arise in response to local stimuli. Although the material studied is not definitive in this regard, it has not yielded a histologic demonstration that there is a generalized deterioration of articular cartilage that undergoes direct transition into osteoarthritis. Even if the degeneration of the articular cartilage is largely of secondary type, its extent and character may be profoundly

* It is to be noted that in the only study dealing quantitatively with this phenomenon in articular cartilage, the old cartilage that had reduced cell density was also affected by osteoarthritis.[19]

† Collins,[2] p. 81.

‡ Bennett and Bauer,[1] p. 44.

affected by a variety of systemic factors.

It may be concluded that the osteoarthritis of mice more closely simulates its human counterpart than do experimental models employed widely in certain other fields of pathological research. Some limitations of the usefulness of mice in this type of investigation have been noted in another respect: The articulations of the small rodents have a major structural difference from those of man in that their epiphyses do not close.§ Furthermore, although there is experimental evidence that certain hormones can affect the articular cartilage of experimental animals adversely, there is no evidence that human osteoarthritis is an expression of any disorder of the endocrine system in man.‖ These matters are dealt with in following reports.¶

Summary and Conclusions

A detailed account of the pathological anatomy of spontaneous degenerative joint disease in 670 laboratory mice, 12 to 22 months old, is presented. Murine osteoarthritis closely simulates its human counterpart in three major respects: (1) its anatomical features, (2) its distribution localized according to postural characteristics, and (3) its occurrence in older age groups. These considerations, the omnivorous dietary habits, short life span, and availability of highly inbred strains, make the mouse a suitable experimental animal for the study of degenerative joint disease.

Dr. T. F. Hilbish and Mr. J. M. Morel, M. T., furnished the roentgenograms, and Dr. M. Potter, many macerated skeletons.

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Natural History of Degenerative Joint Disease in Small Laboratory Animals

2. Epiphyseal Maturation and Osteoarthritis of the Knee of Mice of Inbred Strains

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Articular cartilage may be regarded as a specially differentiated portion of the epiphyseal cartilage. It responds to physiological stimulation as does the epiphyseal cartilage in several respects. This is illustrated in the case of the response to excessive secretion of the growth hormone of the hypophysis in man. When the epiphyseal plate is youthful, such stimulation results in gigantism. When the epiphyses have united, acromegaly results.

The observation of the articular hypertrophy and degeneration in acromegaly * and the demonstration of comparable changes in laboratory animals subjected to similar stimulation † represent the basis for the hypothesis that pathological stimulation of the chondrocytes followed by degeneration of the articular cartilage is the fundamental pathogenetic mechanism accounting for the development of osteoarthritis.‡

These considerations make it of interest to compare the changes that take place with aging in the epiphyseal and articular cartilages. There is a great deal of information concerning factors that affect the behavior of the epiphyses in the rat.[11] In contrast, relatively little is known about the patterns of epiphyseal development in the mouse. The only systematic treatment of epiphyseal union in the mouse is that of Dawson,[12] who found that the pattern was quite similar to that in the rat.[13] More recently, Silberberg and Silberberg [8] have demonstrated that there are variations in the pace at which the epiphyses of the knee of mice of various strains undergo their development. Furthermore, in old mice a degree of closure was found to occur at times.[9] Dawson referred to the failure of the epiphyseal plates to disappear in the rat and mouse as "lapsed union" and regarded it as an inherent characteristic of the epiphyses of these animals. Todd,§ on the other hand, considered lapsed union to be a pathological phenomenon that depended upon some undetermined environmental factors. It has been suggested that differences in the rate of development and senescence of epiphyseal and articular cartilage among various strains of mice are profoundly influenced by the amount of various endogenous hormones to which these tissues are exposed.[7] Ossification and closure of epiphyses are often employed as a measure of "skeletal age" in physiological studies.‖ McCay ¶ has recently expressed his belief "that in an animal where the epiphyses do not seal, as in the rat, there may be some key to aging in the bones."

The great advances that have been made in the genetics of the mouse have provided valuable information and experimental material in other areas of research. The availability of numerous strains already in-

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National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Department of Health, Education, and Welfare.

* References 1 and 2.

† References 3-5.

‡ References 6 and 7.

§ Todd, T. W.: Epiphyseal Union in Mammals, with Special Reference to Rodents, unpublished paper, cited by Zuck.[14]

‖ References 8, 11, 15.

¶ McCay, C. M., in discussion on Medaivar.[10]

bred for these purposes offered an opportunity for a survey of the variation in epiphyseal aging, as measured by resorption of the plate, and arthritis among groups homozygous with respect to many factors already known. Through such a survey, it was hoped to (1) confirm the existence of and identify strains with high and low vulnerability to osteoarthritis; (2) examine the hypothesis that osteoarthritis is an expression of senescence of articular cartilage, and possibly related more generally to skeletal aging, and (3) give insight into the factors operating in the susceptibility of mice to osteoarthritis.

Materials and Methods

The knees of 670 mice 12 to 22 months old were studied histologically. The methods employed and the criteria for diagnosis of osteoarthritis have been described previously.[17] Seventeen of the strains recorded in Table 1 are well known and are listed in the "Standardized Nomenclature for Inbred Strains of Mice."[19] The 18th strain, designated as STR/1N, has been developed in the Inbred Colony of the National Institutes of Health, and its characteristics will be reported elsewhere.# It is a derivative of the STR/N mouse and differs from it in its coat color and in the development of obesity while on a stock diet during the fourth to the fifth month of life. For purposes of comparison of the occurrence of osteoarthritis at older and younger ages, the mice have been divided into two age groups: 12-17 and 18-22 months. The occurrence of osteoarthritis in them is presented in Table 2 and graphically in Figure 1. In each instance, the severity of osteoarthritis represents the average of both knees.

It has been found that a variable degree of closure of the distal epiphysis of the femur and the proximal epiphysis of the tibia takes place during the second year of life. Fusion of the epiphyses develops most rapidly in the central portions of the bones and then proceeds toward the cortex on the lateral and medial aspects. The degree of closure was estimated in that section of the knee in which it was most advanced. It was graded according to the disappearance of cartilage from the combined epiphyseal plates and replacement by bone marrow or trabecular bone:

0: No disappearance of epiphyseal plates (Fig. 2*A*)

±: Minute defects in the epiphyseal plates, but less than one-quarter of the total

3+ :Disappearance of three-quarters of the plates

TABLE 1.—*Strain, Age and Sex of Mice Studied*

Strain	Generation (F)	12-17 mo.				18-22 mo.				12-22 Mo.		Total No.
		Males		Females		Males		Females		Males	Females	
		No.	Av. Age	No.	Av. Age	No.	Av. Age	No.	Av. Age	No.	No.	
A/HeN	91-92	8	14.5	13	14.5	8	19.6	11	20.2	16	24	40
A/LN	90-92	9	16.0	19	14.3	11	19.5	8	19.4	20	27	47
BALB/cAnN	76-79	7	14.7	16	14.4	13	19.7	19	20.0	20	35	55
BL/HeN	52-55	11	14.2	12	14.8	7	20.0	11	20.1	18	23	41
BLCP/N	5-7	3	16.0	7	15.9	8	19.8	11	19.5	11	18	29
BR8UNT/N	43-45	15	13.9	15	15.3	6	19.2	10	19.4	21	25	46
C3H/HeN	28-30	5	13.2	14	13.1	10	20.6	..	....	15	14	29
C3HfB/HeN	17-20	2	13.5	10	15.3	12	20.7	14	20.1	14	24	38
C57BL/6JN	38-39	5	14.4	7	15.3	17	20.2	14	19.8	22	21	43
C57BL/10H-2d	13-15	5	15.2	8	15.1	6	20.5	7	20.6	11	15	26
C57BL/10ScBs	13-15	5	15.6	10	15.0	5	19.6	9	19.6	10	19	29
C57BR/cdJN	80-82	5	14.2	8	14.8	10	20.0	12	20.0	15	20	35
C57L/HeN	82-87	7	15.6	9	15.0	10	20.0	15	20.1	17	24	41
C58/LwN	94-96	14	14.2	10	14.1	..	....	..	....	04	10	24
DBA/2JN	41-43	4	15.5	15	14.6	12	20.3	14	19.4	16	29	35
STR/N	36-38	6	13.3	9	15.2	4	19.0	10	20.1	10	19	29
STR/1N	34-36	7	14.4	12	14.1	8	19.4	13	19.9	15	25	40
SWR/N	47-48	6	15.0	5	14.6	8	20.1	14	19.9	14	19	33
Total		124		199		155		192		279	391	670

Jay, G. E., Jr. paper to be published.

2+: Disappearance of one-half of the plates

3+: Disappearance of three quarters of the plates

4+: Complete disappearance of the plates Fig. 2*B*)

The degree of closure was usually equal in the two knees. The average degree of closure has been computed for each strain in four age groups (12-14, 15-17, 18-20, and 21-22 months). The results are presented in Table 3.

Incidence of Spontaneous Osteoarthritis in Various Strains

It is apparent (Table 2) that there is great variation in the incidence of osteoarthritis, the incidence being as high as 93% in one strain (STR/1N) and as low as 2% in another (A/LN). This offers strong confirmation of the observation by the Silbergs that genetic factors operate in the development of degenerative joint disease in mice. There is some increase in the incidence in the second half of the second year, but in most strains the patterns of degenerative joint disease are manifest at 15 to 17 months of age. The over-all higher incidence observed in males is significant statistically at the $P=0.01$ level. The data provide no evidence that the sex difference depends on age or strain.

TABLE 2.—*Osteoarthritis of the Knee Among Various Strains of Mice*

Strain	Sex	12-17 Mo.						18-22 Mo.					
		Severity of Osteoarthritis						Severity of Osteoarthritis					
		No. Animals	0	1+	2+	3+	4+	No. Animals	0	1+	2+	3+	4+
A/HeN	Males	8	3	5	0	0	0	8	3	4	1	0	0
	Females	13	5	7	1	0	0	11	5	5	1	0	0
A/LN	Males	9	8	1	0	0	0	11	11	0	0	0	0
	Females	19	19	0	0	0	0	8	8	0	0	0	0
BALB/cAnN	Males	7	1	4	1	1	0	13	1	7	4	1	0
	Females	16	10	4	1	1	0	19	7	11	1	0	0
BL/HeN	Males	11	3	4	2	1	1	7	2	2	1	1	1
	Females	12	1	5	3	2	1	11	2	5	2	1	1
BLCP/N	Males	3	3	0	0	0	0	8	6	2	0	0	0
	Females	7	5	2	0	0	0	11	9	2	0	0	0
BRSUNT/N	Males	15	2	7	5	1	0	6	0	1	3	2	0
	Females	15	8	4	2	0	1	10	4	3	1	1	1
C3H/HeN	Males	5	3	1	1	0	0	10	4	5	1	0	0
	Females	14	11	2	1	0	0	--	-	-	-	-	-
C3HfB/HeN	Males	2	1	1	0	0	0	12	4	3	4	1	-
	Females	10	9	0	1	0	0	14	6	6	2	0	0
C57BL/6JN	Males	5	4	0	1	0	0	17	10	7	0	0	0
	Females	7	3	4	0	0	0	14	7	6	1	0	0
C57BL/10H-2d	Males	5	5	0	0	0	0	6	5	1	0	0	0
	Females	8	7	1	0	0	0	7	5	2	0	0	0
C57BL/10ScBs	Males	5	4	1	0	0	0	5	4	1	0	0	0
	Females	10	8	2	0	0	0	9	9	0	0	0	0
C57BR/cdJN	Males	5	1	4	0	0	0	10	4	6	0	0	0
	Females	8	4	4	0	0	0	12	5	5	1	1	0
C57L/HeN	Males	7	7	0	0	0	0	10	1	6	1	0	2
	Females	9	7	2	0	0	0	15	7	5	1	2	0
C58/LwN	Males	14	5	8	1	0	0	--	-	-	-	-	-
	Females	10	7	3	0	0	0	--	-	-	-	-	-
DBA/2JN	Males	4	1	1	2	0	0	12	1	2	4	5	0
	Females	15	3	7	2	3	0	14	1	6	5	2	0
STR/N	Males	6	4	1	1	0	0	4	3	0	0	1	0
	Females	9	5	2	2	0	0	10	8	1	0	1	0
STR/1N	Males	7	0	0	3	3	1	8	0	0	3	1	4
	Females	12	2	1	3	5	1	13	1	3	1	2	6
SWR/N	Males	6	1	2	2	1	0	8	5	1	1	0	1
	Females	5	1	2	2	0	0	14	5	6	2	1	0

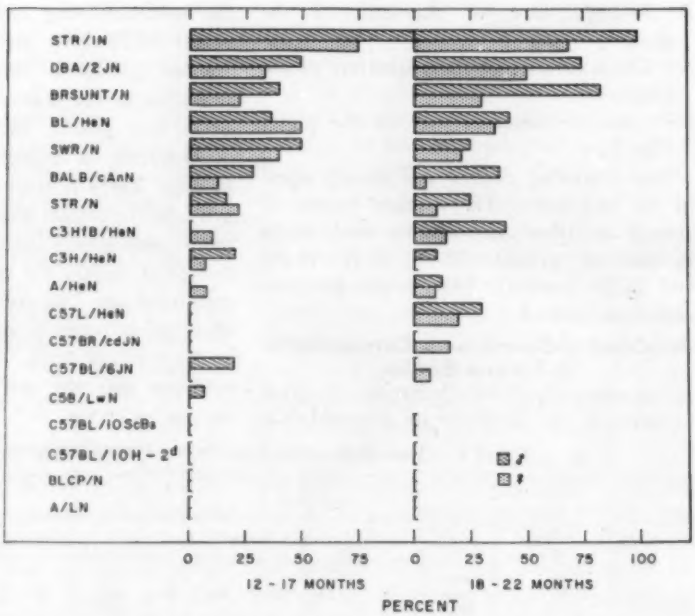

Fig. 1.—Incidence of osteoarthritis of the knee of greater than 1+ severity in 18 strains of mice.

TABLE 3.—*Epiphyseal Closure at the Knee Among Various Strains of Mice**

Strain	Males				Females			
	Age, Mo.				Age, Mo.			
	12–14	15–17	18–20	21–22	12–14	15–17	18–20	21–22
A/HeN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/LN	0.0†	0.1	0.2	0.1	0.1	0.1	0.2	0.1
BALB/cAnN	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
BL/HeN	0.1	0.3	0.3	0.3	0.0	0.1	0.2	0.1
BLCP/N	----	0.3	0.4	0.3	----	0.1	0.6	0.5
BRSUNT/N	0.1	0.1	0.1	0.3	0.0	0.0	0.0	0.1
C3H/HeN	0.0	0.3†	0.5	1.3	0.1	0.2	0.5†	----
C3HfB/HeN	0.3	----	0.7	1.2	0.2	0.3	0.6	0.6
C57BL/6JN	0.0†	0.1	0.6	0.7	0.0†	0.2	0.3	0.4
C57BL/10H-2d	0.3†	0.3	0.5†	0.7	0.3	0.3	0.0†	0.5
C57BL/10ScBs	0.5†	0.5	0.4	0.6	0.2	0.4	0.5	0.5
C57BR/cdJN	0.3	1.0†	3.0	2.8	0.4	1.1	2.3	2.7
C57L/HeN	0.9	1.9	2.3	3.3	0.4	1.0	1.5	1.7
C58/LwN	0.1	0.3	----	----	0.0	0.1	----	----
DBA/2JN	----	0.1	0.5	0.8	0.4	0.4	0.4	0.4
STR/N	0.0	0.0†	0.3	0.0†	0.1	0.0	0.0	0.0
STR/1N	0.0	0.1	0.0	0.1	0.3	0.0	0.1	0.1
SWR/N	0.0	0.2	0.1	0.1	0.0	0.0	0.1	0.0

* Average degree in units of 0 to 4+.
† One animal.

Epiphyseal Closure in Various Strains

Several facts emerge from Tables 2 and 3.

1. Closure of the epiphyseal plates at the knee progresses to a variable degree among the strains during the second year of life. It is virtually complete in some strains and absent in others. To the extent that advanced closure occurs in some strains, the phenomenon of "lapsed union" is less pronounced in mice than in rats.

2. The pattern of closure is quite consistent within a given strain and thus is ultimately under genetic control. The greatest degree of closure of the epiphyses at the knee is achieved by the C57L/HeN and C57BR/cdJN strains. These are closely re-

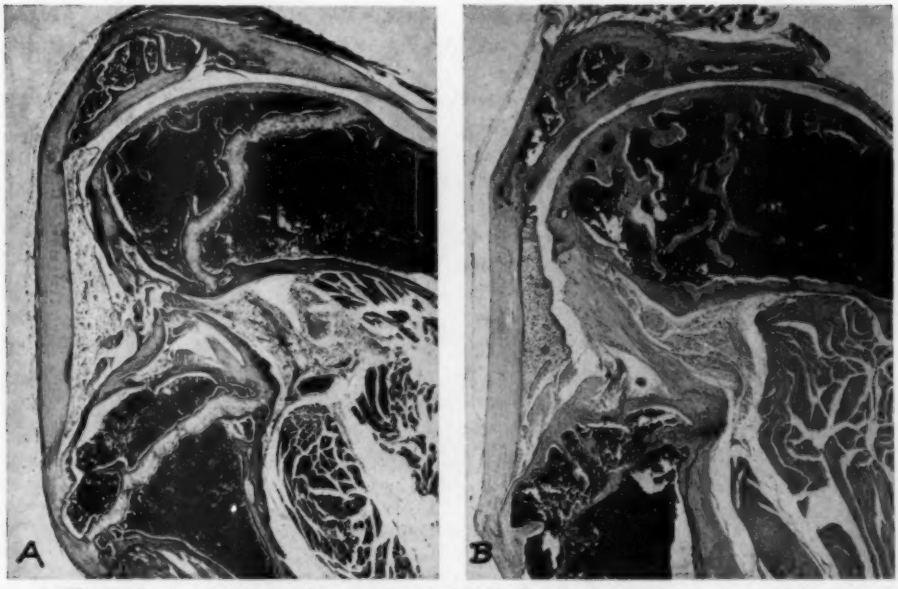

Fig. 2.—Variation in the degree of closure of the epiphyses at the knee. (*A*) No closure is present. (*B*) The distal plate of the femur has disappeared. Only the most dorsal portion of the plate of the head of the tibia persists. This would be classified as 3+ to 4+ closure. Hematoxylin and eosin; reduced to approximately 72% of mag. × 16.

lated to each other, the C57L having arisen from the C57BR line originally. The C57BR mouse, in turn, was derived at an earlier date from the C57BL strain.* Three lines of C57BL mice share, however, a degree of closure that is quite different from the preceding. The related C3H and C3HfB strains also have a common pattern of epiphyseal maturation at the knee. Little advancement of the epiphysis is found in the closely related STR/N and STR/1N mice during the second year.

3. The degree of closure is greater in males than in females.

4. No correlation can be established between the development of osteoarthritis and the progression of epiphyseal closure. If indeed osteoarthritis reflects an aging of articular cartilage, aging of articular cartilage does not parallel that of the epiphysis. It is to be noted that the measure of epiphyseal aging made here differs from that employed by the Silberbergs, inasmuch as they have been concerned with the early and II). Whether there is a correspondence between the early and the late behavior of the epiphysis cannot be stated at present. Data bearing on this are presented in a following study.[18]

Comment

The occurrence of major variations in the incidence of degenerative joint disease among various highly homozygous strains of mice offers an opportunity to evaluate factors that may be involved in its development. All the pertinent differences among the strains are too numerous to be mentioned at this time. It is noted, however, that there is a remarkably high incidence of osteoarthritis in strain STR/1N as compared with that of STR/N. These strains

* Heston, W. E.: Development of Inbred Strains of the Mouse and Their Use in Cancer Research, in Roscoe B. Jackson Memorial Laboratory 20th Commenoration Lectures on Genetics, Cancer, Growth, and Social Behavior, given in honor of Dr. C. C. Little, printed by the *Bar Harbor Times*, Bar Harbor, Maine.

are closely related genetically and have similarly little epiphyseal closure. An outstanding difference is the occurrence of obesity in the STR/1N mice. A high incidence of osteoarthritis has been found by Silberberg and Silberberg [7] in mice of some strains maintained on a high-fat diet. In C57BL mice this could not be correlated with obesity, and it was suggested that the excessive dietary fat might be utilized metabolically by the articular chondrocytes.[7] In experiments with mice of the Y′ strain, a positive association could be established between the arthritis and the obesity.[10] It has long been a clinical impression that obesity aggravates osteoarthritis in man, although this does not appear to be true in the case of Heberden's nodes of the fingers.[20]

Brief comment must also be made about the role of the thyroid gland in these strain differences. Variation is known to exist in the structure and function of the thyroid gland among some strains.† The lack of correlation between epiphyseal closure and osteoarthritis suggests that these differences in the thyroid gland do not play a dominant role either in governing the variations in epiphyseal maturation or in protecting articular cartilage from degenerative joint disease.

No correlation can be established between the progression of epiphyseal closure or of osteoarthritis and the longevity of the strains or the onset of sexual maturity so far as this can be gauged by the age at which pregnancy is first observed.

Summary and Conclusions

The occurrence of osteoarthritis and the progression of epiphyseal closure has been studied in the knees of 670 mice 12 to 22 months old. They comprised 18 inbred strains. Enormous variation in the incidence of osteoarthritis has been found among the strains. The degree of fusion of the epiphyses is influenced genetically, and usually is greater in males than females. No correlation can be established between the development of osteoarthritis and skeletal aging as measured by epiphyseal union.

† References 7, 21, 22, 23.

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Natural History of Degenerative Joint Disease in Small Laboratory Animals

3. Variations in Epiphyseal Maturation in the Head of the Femur Among Inbred Strains of Mice

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In the course of surveying the distribution of degenerative joint disease, an apparently anomalous development of the epiphysis of the head of the femur was encountered in a number of mice late in the second year of life. In some instances, no secondary center of ossification had appeared (Fig. 1*A*), while in some others complete ossification and union of the epiphysis had taken place (Fig. 1*C*). Furthermore, the degree of development was

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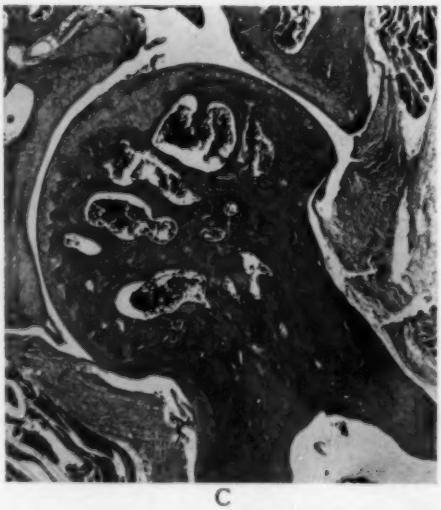
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Department of Health, Education, and Welfare.

B

A



C

Fig. 1.—*A*, head of femur of DBA/2JN mouse, 19 months old. No secondary center of ossification is present. *B*, contralateral head of same mouse. A secondary center is present. The epiphyseal plate has not disappeared. *C*, head of femur of C3H/HeN mouse; showing complete ossification and closure of the epiphysis. Hematoxylin and eosin, reduced to 88% of mag. × 20.

not always equal in the hips of the same animal (Fig. 1*B*). This phenomenon has not been described previously. It has been investigated systematically to determine whether this variation reflects a local autonomy of ossification of the epiphysis or whether it is subject to factors that govern maturation of epiphyses elsewhere in the body.

Methods and Materials

Serial sections of the head of each femur of 147 mice, 20 to 22 months of age, were examined. The number of animals in each strain and the sex are indicated in Figure 2. The colony of animals and the technical methods have been described previously.* The degree of epiphyseal maturation was graded as follows:

0: No secondary center of ossification present
1+: Incomplete secondary center of ossification
2+: Complete ossification of secondary center; no closure of the plate
3+: Complete ossification of the secondary center; incomplete closure of the plate.
4+: Complete ossification and closure of the epiphysis

* References 1 and 2.

Results

The average degree of maturation was computed for males and females of each strain, and the results are presented graphically in Figure 2. The degree of maturation of the epiphysis usually was symmetrical, but in 20% of the animals there was a difference of as much as 2+ from side to side.

The degree of development of the epiphysis of the head of the femur was found to be subject to genetic influence and varied greatly between the sexes. In the C57L/HeN, C57BR/cdJN, C3H/HeN, and C3HfB/HeN mice complete ossification and epiphyseal union had occurred in all animals examined. In other strains the ossification and closure of the epiphysis was complete in male but not in female mice. In still other strains little ossification of the epiphysis was found in males or females.

An attempt to correlate these changes with those in the epiphyses of the knee is included in Figure 2. In this is charted

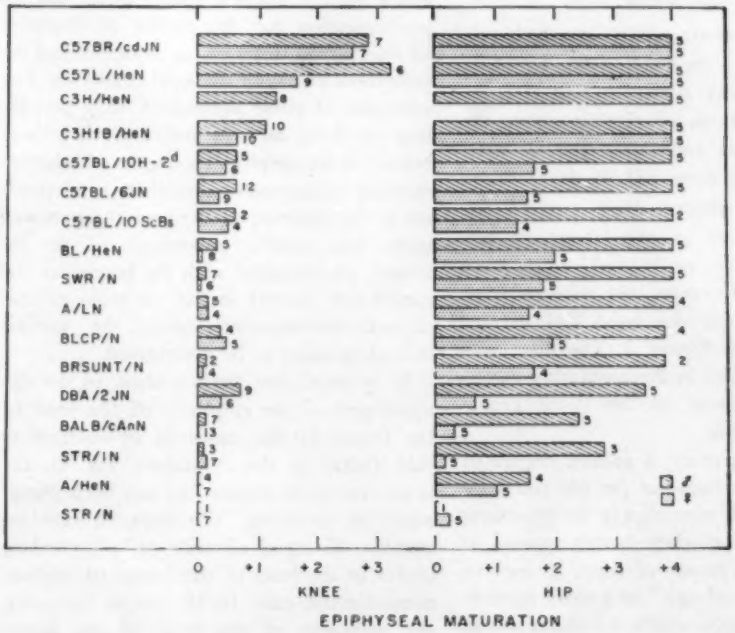


Fig. 2.—Epiphyseal maturation of the hip and knee of mice 20 to 22 months old. The numerals at the right of each bar represent the number of animals examined in each group.

the degree of closure of animals 20 to 22 months old, graded as described in a preceding report.[1] In general, the ultimate development of the epiphyses in the head of the femur and those at the knee correspond fairly closely. The correlation (P is well below 0.01) of advanced closure in the two groups is significant statistically. The correlation ($P=0.15$) between the lower degrees of epiphyseal maturation at the hip and at the knee are suggestive but not conclusive statistically. At the knee, as well as at the hip, "aging" of the epiphysis is less complete in females than in males.

Comment

The only previous report in which epiphyseal union of the head of the femur of the mouse has been studied is that of Dawson.[3] Three patterns of closure among the various epiphyses were observed by him: 1. Those uniting early (21-23 days) do so completely. In general they parallel the sequence observed in the laboratory rat. 2. Varying degrees of "lapsed" union were found in the olecranon, the tuberosity of the calcaneus, and the head and greater trochanter of the femur. 3. The plate was never found united in the remaining epiphyses, such as those of the distal femur and proximal tibia.

In the present study, a greater degree of closure has been found at the hip and knee in some groups of mice than in the preceding study. When a secondary center appears in the head of the femur of mice, it does so at about 15 days of age.[4] In a small number of C3H/HeN mice which we examined at a younger age than those included in the present series, complete ossification and closure of the epiphysis of the head of the femur was found; in some instances, this was in mice as young as 12 months. There appears to be a correlation between the ultimate development of the late-closing epiphysis in the knee and that of the epiphysis of the head of the femur, which matures at some earlier time. To the extent that this is true, the factors that govern closure late in life may well be presumed to have been operating at an earlier period as well. Variation in the epiphyseal closure of the distal femur and proximal tibia that becomes manifest during the second year of life, accordingly, would not likely reflect a deviation of hormonal activity arising in the second year. On the other hand, it has been reported that major structural differences in the thyroid gland among various strains of mice become prominent in the older age group.[5]

Johnson[4] observed that primary ossification centers appeared sooner on the right side than on the left side of the mouse at times. In the present study, there was no predisposition for the earlier development of one side in instances of asymmetrical development of capital femoral epiphyses. The existence of some asymmetry in a proportion of these animals indicates that there resides in the epiphyses a degree of inherent capacity to become ossified that is independent of the systemic endocrine and nutritional state. The relative importance of the intrinsic, as compared with the hormonal and nutritional, factors in the variation of epiphyseal development among the various strains remains to be determined.

It is noted that the variation in the development of the epiphysis of the head of the femur of the mouse is in contrast to that found in the laboratory rat. In the latter, complete closure has not been recognized as occurring. We have, in rare instances, observed absence of a secondary center in the head of the femur of mature, nonpedigreed rats. In 10 Syrian hamsters, the epiphysis of the head of the femur has been found closed during the second year, but closure was not seen in several guinea pigs 2 to 3 years of age.

Summary and Conclusions

A histological study of the head of the femur of 147 mice, 20 to 22 months old, has revealed great variation in the degree of epiphyseal differentiation. The variation ranges from complete absence of a secondary center to complete ossification and

closure of the epiphysis. The degree of development is genetically influenced and is more advanced in males than in females. The degree of closure of the epiphyses at the knee can be correlated generally with the maturation of the epiphyses of the head of the femur.

Mr. Paul Snow gave valuable assistance during this study.

Mr. Jerome Cornfield made the statistical analysis of the data.

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Natural History of Degenerative Joint Disease in Small Laboratory Animals

4. Degenerative Joint Disease in the Laboratory Rat

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The laboratory rat would offer advantages over the mouse in the study of degenerative joint disease if it could be demonstrated that it is susceptible to the lesions. Aside from the greater suitability of the rat for a variety of nutritional and endocrine investigations, the greater size of the articular structures would allow easier evaluation and sampling of the lesions. The only previous investigation of the joints of older rats is that of Saxton and Silberberg,[1] who examined the knees in animals up to 620 days of age. Aging changes, in the form of proliferation and degeneration of chondrocytes, were found in some of these.

Materials and Methods

A series of 144 rats between 21 and 30 months of age was studied. They were of eight inbred strains. In all but a few instances they were retired breeders. With the exception of 22 animals, they were maintained in the same room and under the same conditions as the mice already described.[2] These 22 animals were of the same strains as the remainder and were maintained under comparable conditions after being transferred from the Inbred Colony.* Details concerning the strains, numbers, and ages of the rats are presented in the accompanying Table. In addition to these rats, 13 stock rats (Sprague-Dawley), 24 months old, were studied histologically. Skeletons of eight rats,* 27 to 33 months of age, were macerated by the papain technique. All animals received Purina Chow.

Histological examination was made routinely on the right knee and ankle. In the case of the knee, the sections were like those made in the mice. Three coronal sections were made of the tibiotarsal region and included the tibiotalar and talocalcaneal articulations.

Results

Two types of degenerative changes were found in the articular cartilages:

1. The changes of osteoarthritis, like those already described in the mouse.

2. A localized cystic lesion that did not progress to articular erosion. In its initial stages, this lesion was characterized by dis-

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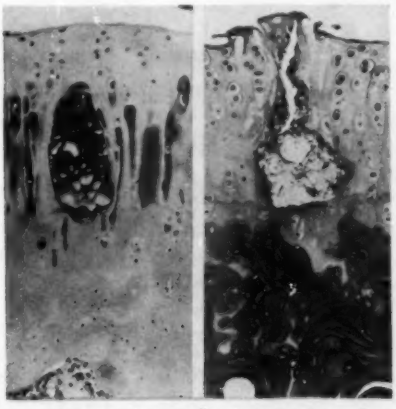
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Department of Health, Education and Welfare.

* Drs. K. C. Snell, H. L. Stewart, and E. Streicher furnished these animals.

Strain, Age and Sex of Inbred Rats Studied

Strain	Generation (F)	21-23 Months		24-26 Months		27-29 Months		30 Months +		21-30 Months		Males & Females Combined
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	
Albany	3-5	1	3	5	4	3	0	0	0	9	7	16
AxC 9035	44-46	2	7	5	6	1	5	0	2	8	20	28
Buffalo	16-17	1	3	5	7	0	6	0	0	6	16	22
Fischer	55-57	4	5	2	5	0	0	0	0	6	10	16
M-520	58-59	1	0	5	5	3	5	0	0	8	10	19
O'Grady	36-37	0	1	2	0	0	0	0	0	2	1	3
O-M/N	14-16	5	3	5	6	0	0	0	0	11	9	19
Wistar	19-20	3	5	5	5	0	3	0	0	8	13	21
		17	27	34	38	7	19	0	2	58	86	144

solution of the articular cartilage into an ovoid, chondromucoid puddle (Fig. 1*A*). It usually was no greater than approximately 50μ in diameter and was located in the deep



A, early chondromucoid degeneration of articular cartilage of rat. Azure A-eosinate; × 115. *B*, cystic degeneration of articular cartilage of rat. Hematoxylin and eosin; × 115.

portion of the articular cartilage. The material within the cyst was metachromatic when stained with azure A-eosinate. In the more advanced stage, the mucoid material had disappeared from the cyst, and the lesion contained only a few delicate, eosinophilic fibers, which were arranged haphazardly. The fibers were not argyrophilic (Wilder's stain) and were yellow in the Van Gieson preparation. At the apex of the cyst a minute perforation through the articular surface could often be seen (Fig. 15). One or more of these cysts might be present in an individual joint.

Osteoarthritis was present in the knee of 3 of the 157 rats examined. Two of these were males of the O-M/N strain; the other was an M-520 female, 27 months old. The lesions were of Grade 1 severity, according to the criteria described in the study of mice. By way of contrast, an appreciable degree of osteoarthritis was present in either the tibiotalar or the talocalcaneal joint of the majority of the animals. It was somewhat severer in the O-M/N, Buffalo, and Albany strains than in the others. The changes were best developed in the ventral portions of these joints, but did not lead to serious deformity. In the severer grades, mild synovitis detritica appeared in these joints, and the adjacent tendon sheaths became somewhat swollen with mucoid fluid. This rarely became sufficiently pronounced to be recognized grossly. It is to be noted that decubitus ulcers, usually found in the plantar region of old rats housed in cages with wire-mesh floors, were not present in these animals. Cystic degeneration was rarely present in the ankle.

Cystic degeneration of the articular cartilage was found in 29 of the 157 knees examined. Because of the small size of the lesions, the true incidence cannot be ascertained unless serial sections or gross examination be made. It was present in 9 of 19 O-M/N rats sectioned casually. This was the strain most prone to develop osteoarthritis.

A histological survey of all the joints of one rat (AxC 9935, 30 months old, female) was made by serial section. It revealed cystic degeneration in the costovertebral, as well as the knee, joints.

Degeneration of the articular cartilages of the small bones of the extremities, in the form of loss of viable chondrocytes and depletion of metachromatic material from the matrix, occurred in a manner similar to that of the mouse. Similar, but much less conspicuous, changes were present in the knee as well. The articular chondrocytes of the older rat often had a semilunar arrangement of the nucleus about a hyalinized cell mass. This is uncommon in the mouse.

Small areas of fusion of the epiphysis of the distal femur and proximal tibia were present in several of the strains. They were most conspicuous in the O-M/N rats and, to a less extent, in the AxC 9935 strain. The attempt at epiphyseal closure was somewhat greater in the males than in the females. In one of the macerated preparations, a small area of erosion was found at the internal margin of the medial condyle of one knee. Otherwise, there were no mar-

ginal osteophytes or other stigmata of degenerative joint disease.

Comment

The various strains of rats displayed a marked resistance to the development of osteoarthritis of the knee. They also were subject to much less variation in or progression of epiphyseal maturation than the mouse. The reason for the apparent lack of vulnerability of the knee of the rat to osteoarthritic erosion is not forthcoming. It may be that the joint is subjected to less trauma than is that of the mouse, since the latter is more active and has more opportunity within the confined space of the cage to drop from a height, e.g., the food hopper or water bottle.

Moffett [3] found that there was no alteration in the ability of Diodrast to diffuse through the synovial membrane of the knee of Wistar rats 20 to 24 months old as compared with those 2 to 3 months old. The preservation of the ground substance of the soft tissues of the aging rat knee coincides with the present observation of the relative stability of the matrix of its cartilage.

In view of the limited occurrence of osteoarthritis in the rat, this species appears less suited than the mouse for experimental investigation of the pathogenesis of osteoarthritis.

Summary

Joints from 157 rats between 21 and 30 months of age have been studied histologically. They comprised eight inbred and one stock strain.

The rat appears to be resistant to the spontaneous development of osteoarthritis. A pattern of cystic degeneration of articular cartilage is seen more frequently.

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Radiologic and Pathologic Bone Changes Associated with Urticaria Pigmentosa

Report of a Case

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Although urticaria pigmentosa has long been recognized as a dermatologic entity, associated roentgenologic bone changes have been described only in recent years. None of the reported cases had pathologic studies of bone.

In 1949, Ellis,[1] in reporting the case of an infant who died in the active stage of urticaria pigmentosa, described mast cells in large numbers in the bone marrow and viscera; radiographs of bones showed no abnormality.

In 1952, Sagher, Cohen, and Schorr[2] reported, in a patient with urticaria pigmentosa, changes which were described as generalized cystic osteoporosis of the ribs, stippling of the bones of the skull, and generalized myelosclerosis of the vertebrae and pelvic bones. Bone biopsy was not performed, but sternal puncture revealed no abnormality; mast cells were not found.

In 1952, Clyman and Rein[3] found roentgenologic evidence of bone changes in two out of eight cases of urticaria pigmentosa; in 1953, Asboe-Hansen[4] reported an additional case. These three cases differed in characteristics among themselves and differed from the original description of Sagher and associates.

Since the radiologically observed bone lesions have not been studied histologically, we are reporting in some detail the radiologic and histopathologic findings in a typical case of urticaria pigmentosa.

Submitted for publication March 26, 1956.

From the Departments of Pathology, Radiology, and Dermatology, DeGoesbriand Memorial Hospital and the University of Vermont College of Medicine.

Report of Case

Early in 1955, the patient, an unemployed white farmer, aged 54, presented himself to the Tuberculosis Clinic of the DeGoesbriand Memorial Hospital because of pain in the right anterior chest of a few weeks' duration. At the age of 13 the patient had been wounded in the right leg with a pitchfork, and subsequent to this a more or less diffuse cutaneous eruption had appeared. The eruption gave few subjective symptoms and had remained essentially unchanged since that time. Except for occasional backaches and recent pain in

Fig. 1.—Maculopapular eruption of urticaria pigmentosa.

the chest, the patient had been in fairly good health.

On physical examination, there was noted a widespread maculopapular eruption, the distribution being often in groups, with individual lesions varying in size from 3 to 6 mm. (Fig. 1). Many of the lesions were telangiectatic, and, upon rubbing, the macules became wheals. The head was spared, but the neck and trunk were severely involved. The upper and lower extremities, with the palms of the hands and the soles of the feet excepted, were covered with lesions. Below the knees the eruption was not quite so severe. No palpable lymph nodes were found, and, except for the skin lesions, the physical examination of the abdomen was within normal limits.

Erythrocyte, leucocyte, and differential cell counts were within normal limits. Serum calcium was 5.0 mEq. per liter; phosphorus, 3.6 mg. per 100 cc., and alkaline phosphatase, 6.0 Bodansky units per 100 cc.

A dermatologic diagnosis of the adult type of urficaria pigmentosa was made.

Radiologic Changes.

The radiologic bone changes were generalized and for the most part symmetrical in distribution, the most striking being in the humeri, femora, pelvis, lumbar vertebrae, and ribs. The most extensive changes in the long bones were in the metaphyses, and the humeri showed the greatest changes (Fig. 2). The individual trabeculae were thickened and decreased in number, and the intertrabecular spaces were widened, resulting in a netlike appearance. The innominate bones (Fig. 3) presented the same pattern except in the iliac fossae, where there was a striking decrease in density. The trabeculae of the vertebral bodies (Fig. 4) were more compactly arranged than elsewhere and were indistinct in outline, the over-all effect being that of slightly increased density. The spinous processes were more osteoporotic than usual. The ribs presented multiple small punched-out areas of rarefaction, similar to changes seen in multiple myeloma or in metastatic malignant disease. The skull and the bones of the hands and feet were considered radiologically normal. The proximal portion of the shaft of the left tibia (Fig. 5) showed a defect in tubulation.

Fig. 2.—Proximal humerus showing thickened trabecular pattern with widened intertrabecular spaces of apparent decreased density.

Fig. 3.—Markedly decreased trabecular pattern and density in the iliac fossae but thickened trabecular pattern throughout the remainder of the bony pelvis.

Fig 4.—Trabecular pattern of the vertebral bodies indistinct in outline. Over-all effect is that of slightly increased density. Incidental hypertrophic changes also noted.

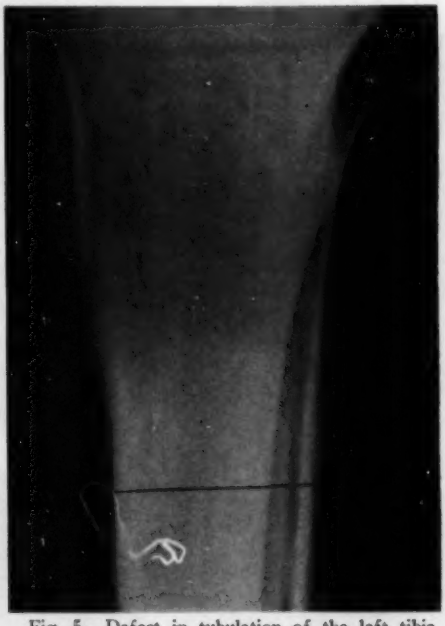

Fig. 5.—Defect in tubulation of the left tibia. This defect was not present in the right tibia.

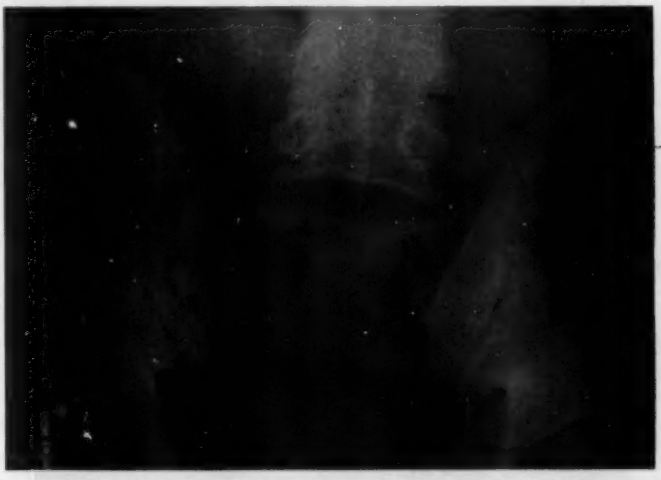

Fig. 6.—Radiograph of pelvis taken in 1951, demonstrating the symmetry of lesions and the same degree of involvement as present radiographs.

Review of radiographs of the lumbar spine taken in 1948 and again in 1951 revealed the same lesions as described above (Fig. 6). So far as could be determined from these three examinations, there had been no change in the appearance of the bony structures in the lumbar region between 1948 and 1955.

Biopsies were performed of the skin and of a rib.

Pathologic Changes.

Several pieces of bone from the rib were removed, fixed in Zenker-formol and 10% formalin, and decalcified with trichloroacetic

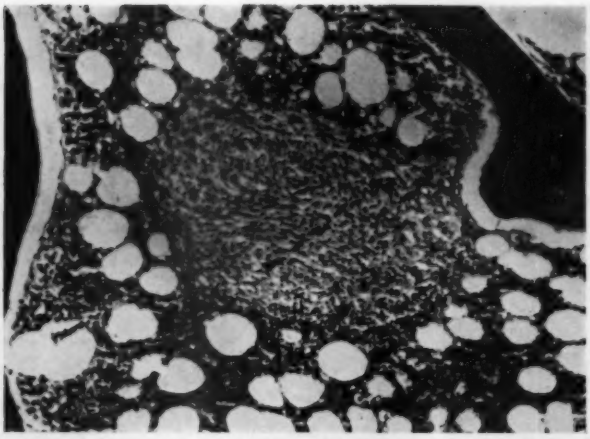

Fig. 7.—Bone marrow containing a well-defined granuloma. Hematoxylin and eosin; × 120.

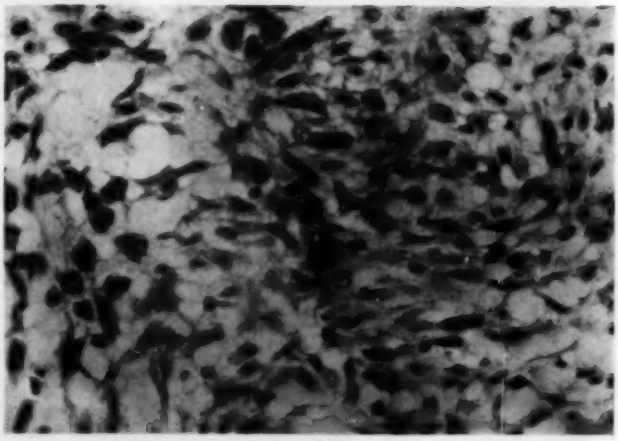

Fig. 8.—Cellular components of the lesion. The majority of cells are spindle-shaped or stellate with fine cytoplasmic processes. Giemsa-Wolbach stain; × 475.

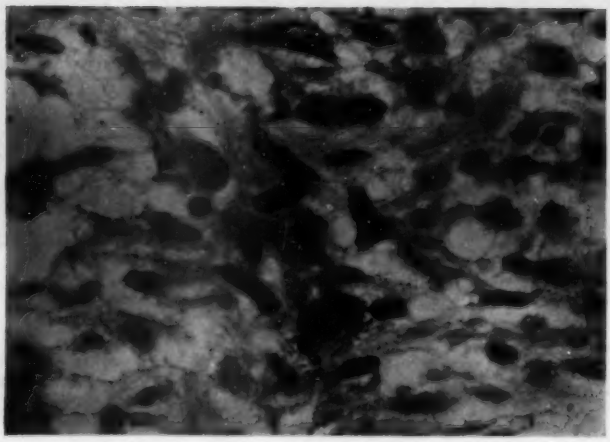

Fig. 9.—The metachromatic granules of the mast cells. Many of the smaller stellate and elongated cells contain fainter staining and fewer granules. Giemsa-Wolbach stain; × 950.

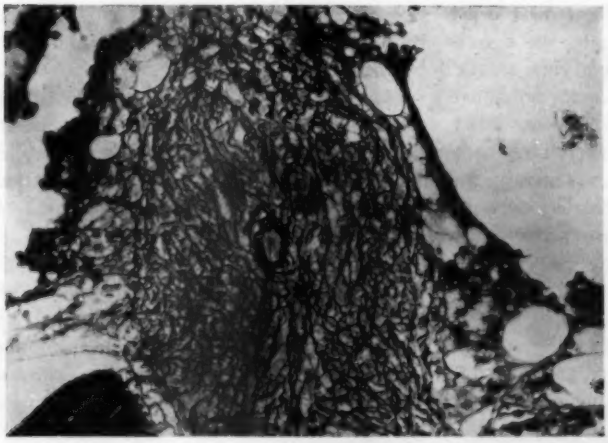

Fig. 10.—Moderately rich reticulum framework freely ramifying among the cell groups. Laidlaw's reticulum stain; × 200.

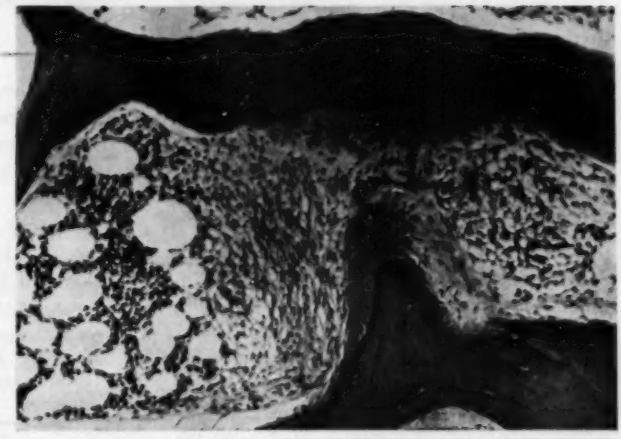

Fig. 11.—Deposition of osteoid seams upon a bone trabecula in association with one of the granulomata. Hematoxylin and eosin; × 125.

acid. Hematoxylin and eosin, Giemsa-Wolbach, toluidine blue, trichrome, and reticulum stains were employed.

Sections of the bone revealed a fair preservation of the osseous and marrow architecture. The outstanding abnormality was the presence of numerous nodular, distinctly granulomatous lesions within the marrow spaces, the lesions varying in size from a collection of a few cells to that of one-half a low-power field (Fig. 7). Occasionally, one of the larger lesions showed resorption of the adjacent bony spicules, with a scalloped, irregular edge without osteoclasts.

The cells which made up the bulk of these lesions were slender, spindle-shaped, or stellate in appearance (Fig. 8). The nuclei were round or, more frequently, elongated, with lightly staining chromatin. Among the cells, a delicate fibrillary network was present, which appeared to merge with the cell cytoplasm. These cells showed relatively little variation in size and nuclear staining. A few eosinophiles were present in most of the lesions. With hematoxylin-eosin staining, mast cells could not be definitely identified. In Giemsa-Wolbach and toluidine blue preparations, however, a large proportion of the cells contained a varying number of fine basophilic cytoplasmic granules and had the typical appearance of mast cells (Fig. 9). There were many transitional forms between the

spindle-shaped cells and typical mast cells.

Stains for reticulum brought out a rich reticulum network freely ramifying among the cell groups but not around individual cells (Fig. 10). The trichrome stain showed fine cellular processes joining each other. Collagen fibers were relatively inconspicuous, and in the smaller lesions were entirely absent. However, around some of the larger lesions, a moderate condensation of collagen was observed, which occasionally merged imperceptibly with osteoid seams upon the bony spicules (Fig. 11).

None of the lesions revealed any evidence of necrosis, nor were multinucleated giant cells seen.

Sections of the skin showed separation of the collagen bundles by edema, increased vascularity of the upper dermis, and round-cell infiltration with numerous mast cells.

Comment

As yet, reported cases of urticaria pigmentosa with bone changes are too few to permit the description of a characteristic pattern. Nevertheless, it is interesting to note the close similarity in the radiographic findings between Sagher's case and our own, whereas the other reported cases appear to differ among themselves, as well as from Sagher's.

The symmetrical involvement of bones in the present case was interrupted only by the defect in tubulation of the left tibia. It will require further investigation to determine whether this disturbance in the growth of the bone is related to this condition.

The histologic changes of bone resorption and bone deposition, although slight, tend to corroborate the radiologically observed findings of decreased bone density, on the one hand, and increased thickening of trabeculae, on the other.

Mast cells are believed to be one of the normal constituents of bone marrow.[5] However, their accumulation as part of distinct granulomata has not, to our knowledge, been previously described and is interpreted as pathologic.

That urticaria pigmentosa may be at times a systemic affliction was suggested by Ellis.[1] Similarly, lymph-node infiltration by mast cells is illustrated by Allen.[6] Hissard and associates,[7] in a case of mast-cell tumor of the skin, found on splenic puncture a large percentage of mast cells. The case herein reported also demonstrates that organs other than the skin may be involved in urticaria pigmentosa.

The absence of hemorrhagic manifestations or coagulation defect in patients with urticaria pigmentosa has been noted by many authors, despite strong presumptive evidence that mast-cell granules contain heparin. The patient in this report likewise showed no abnormal bleeding tendency.

Summary

A case of long-standing urticaria pigmentosa of the adult type with marked bone changes is presented. Because the observed histologic lesions of bone have not been reported previously, they are described in some detail. As these have a distinctly granulomatous appearance with numerous mast cells, the designation of mast-cell granuloma of bone is suggested.

Drs. Robert W. Coon and A. Bradley Soule Jr., Chairmen, Departments of Pathology and Radiology, University of Vermont College of Medicine, made helpful suggestions in the preparation of the manuscript.

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Histological Changes Produced by Injections of Polysaccharides

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The use of partly hydrolyzed, low-polymer dextran (about 80,000 molecular weight) as a plasma-volume expander is quite widespread and might become even more so in case of a world catastrophe. In such an event the doses used per single patient would have to be rather great, and therefore the study of changes produced by the injection of such doses is of practical importance.

It has been shown that both low-polymer and high-polymer neutral polysaccharides have an effect on the morphology of granulation tissue, while the high polymers inhibit the acute inflammatory response, as well as the formation of granulation tissue.* In view of these facts, it was considered interesting to study the reactions of the internal organs of mice to injections of high doses of the native (high-polymer) and partly hydrolyzed (clinical) polysaccharides. This report deals with the changes occurring in mice injected with high- and low-polymer dextrans and levans studied by histological and histochemical means.

Experiments on the Histochemical Behavior of Levans and Dextrans

Samples of levans and dextrans of different degrees of polymerization (levans ranging between molecular weights of 10,000,000 and about 3000, and dextrans between 10,000,000 and about 70,000), supplied by Dr. S. Hestrin, of the Laboratory of Microbiological Chemistry, Department of Biochemistry, and of glycogen (Nutr. Biochemicals Corp.) were used. Glass slides were divided into many squares by a diamond pencil, and a small amount of each of the compounds was put between two layers made by discrete drops of dilute Parlodion (pure collodion). Each slide, containing all the compounds, was put into ethanol 70% to harden the Parlodion before staining. This method was adopted after efforts to make the compounds stick to the slides by mixing them with bovine albumin, human serum, and serum fractions had often resulted in failures because of desquamation.

The slides were treated (*a*) by the usual periodic-acid-Schiff (PAS) technique[3]; (*b*) by the same technique, but with the modification that the periodic acid was dissolved in 70% ethanol,[4] and (*c*) by the PAS method suggested by Mowry, Longley, and Millican,[5] in which both the periodic acid and the leucofuchsin were dissolved in 70% ethanol.

The pertinent results of these experiments are summarized in Table 1. It can be seen that only the high-polymer polysaccharides can be stained by aqueous PAS. As has been shown by Mowry, Longley, and Millican,[5] and independently by Persson,[4] the dextrans can be stained by aqueous leucofuchsin after oxidation by periodic acid in 70% ethanol. The same holds true for the high-polymer levans. The low-polymer levans stain less intensely as the degree of polymerization decreases. All the polysaccharides can be stained by the (*c*) procedure, in which all the steps are done in 70% ethanol. These facts indicate that the products of periodic oxidation of the low-polymer levans are easily soluble in water and can be stained only by the all-ethanol procedure.

Experiments on Mice

The organs taken for histological examination belonged to the mice of Experiment 3 mentioned in the preceding article.[2] Briefly, these 50 mice were divided into five groups. Group A mice were given clinical dextran; Group B, native dextran; Group C, clinical levan, and Group D, native levan. Group E mice were not given polysaccharides and served as controls. The polysaccharides were injected intraperitoneally, 15 mg. per dose, every 12 hours, the last dose having been given about eight hours before the animals were killed. All animals had a small incision made in their backs and were given a daily subcutaneous dose of 500

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TABLE 1.—*Staining of Levans and Dextrans by Different PAS Procedures*

	Levans					Dextrans				Glycogen
	Mol. Wt. $>10^7$	Mol. Wt. 120,000	Mol. Wt. 80,000	Mol. Wt. 60,000	Mol. Wt. 25,000	Mol. Wt. $>10^7$	Mol. Wt. 200,000	Mol. Wt. 80,000	Mol. Wt. 60,000	
Ordinary (aqueous) PAS	+++	+	±	0	0	+++	+++	+	+	++++
Periodic acid in 70% ethanol, aqueous Schiff's leucofuchsin	++++	++	++	±	±	++++	++++	+++	+++	++++
Periodic acid in 70% ethanol, leucofuchsin in 70% ethanol	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

units of penicillin. Further particulars about the polysaccharides and animals are mentioned in the preceding article.

Pieces of lung, liver, spleen, and kidney of the mice were fixed (5-10 minutes after the animals were killed by ether) in Carnoy's fixative. Paraffin blocks were made and 6μ-thick sections were cut. The sections were stained with hematoxylin and eosin and the three varieties of the PAS procedure mentioned above. The sections which were stained by the ethanolic PAS methods were mounted on glass slides by floating them on warmed 70% ethanol. In some slides study of intracellular PAS-positive material was aided by a digestion test with saliva.[8]

Microscopic Findings

Clinical Dextran.—In the animals given clinical dextran no pathologic changes could be seen in sections stained by hematoxylin and eosin in any of the organs. In the liver (Fig. 1) there was no increased amount of glycogen, and most Kupffer cells appeared normal; a few appeared somewhat swollen. With both the alcoholic PAS ing granules in the liver, spleen, lung, and kidney. Most of the granules were found lying free within blood vessels and between cells, but some appeared within the lumina and epithelial cells of the renal tubules, within Kupffer cells in the liver, within septal cells in the lungs, and within the littoral cells of the spleen.

Native Dextran.—In the animals given native dextran conspicuous changes could be seen in the hematoxylin-eosin sections of the liver and spleen. In the liver (Fig. 2) the Kupffer cells were swollen, appeared foamy, and in many instances seemed to obstruct the sinusoidal lumina. In some animals, especially around the portal spaces and near the capsule, the hypertrophied Kupffer cells seemed to have proliferated and appeared like a foam-cell tissue invading the

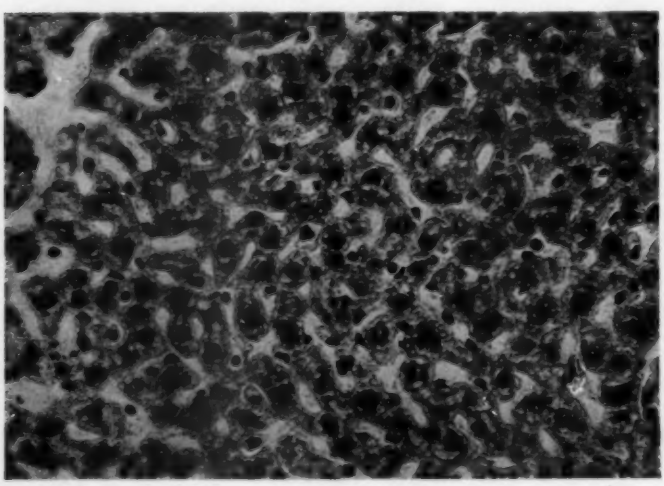

Fig. 1.—Mouse given twice daily 15 mg. of clinical dextran for seven days. Liver. Normal appearance with slight swelling of occasional Kupffer cells. Reduced to 92% of mag. × 370.

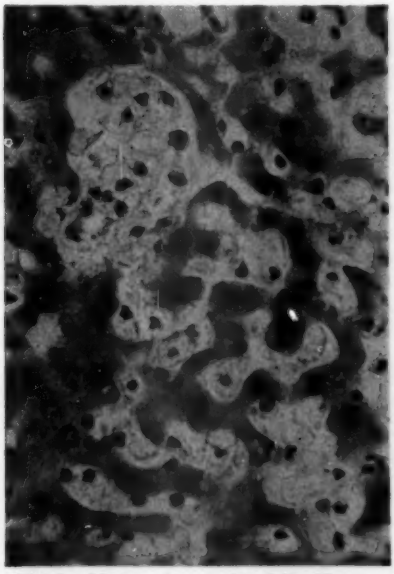

Fig 2.—Mouse given twice daily 15 mg. of native dextran for seven days. Liver. Extreme swelling of Kupffer cells, which appear vacuolated and foamy. In left upper part of Figure, proliferation of the foamy cells with an appearance reminiscent of xanthomatous change. Compression of liver cells. × 370.

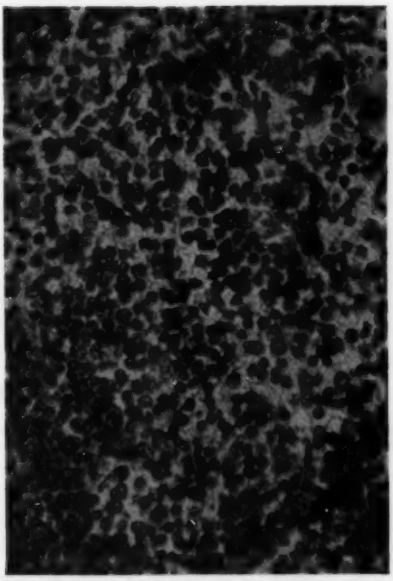

Fig. 3.—Mouse given twice daily 15 mg. of clinical levan for seven days. Spleen. Normal appearance. × 370.

liver. The liver cells appeared compressed, thinned, and rather basophilic. In the spleen, the red pulp contained a variable number of swollen, foamy histiocytes, similar in appearance to the Kupffer cells. No constant changes were observed in the hematoxylin-eosin sections of the lungs and kidneys. Aqueous PAS staining did not reveal any unusual stainable material in any of the organs. With the ethanolic PAS methods, granules, similar to those found in the animals treated with low-polymer dextran, were found in most animals.

Clinical Levan.—In the hematoxylin-cosin sections of the animals treated by clinical levan, the appearances of the spleen (Fig. 3), kidney, and lung tissues were similar to those observed in the animals treated by clinical dextran. In the liver, the Kupffer cells were also similar: either of normal appearance or slightly swollen. The liver cells, on the other hand, appeared swollen, with vacuolated granular cytoplasm and dis-

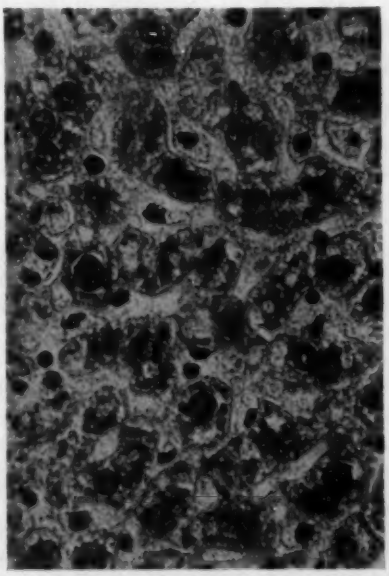

Fig. 4.—Mouse given twice daily 15 mg. of clinical levan for seven days. Liver. Vacuolated granular liver cells with distinct cell borders. Slight swelling of occasional Kupffer cells. × 560.

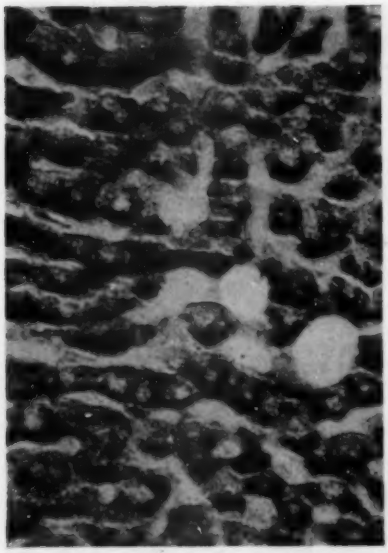

Fig. 5.—From the same preparation as that in Figure 4. Liver cells rich in glycogen. PAS staining; × 370.

tinct cell borders (Fig. 4). The content of these cells stained like glycogen with aqueous PAS (Fig. 5) and could be digested with saliva. Except for this increase in "glycogen," no other unusual constituent could be detected in any organ by any of the PAS methods.

Native Levan.—The livers of the mice treated by native levan exhibited changes which were similar, but severer, than those encountered in the mice treated by native dextran. The liver cells (Fig. 6) were greatly compressed by the hyperplastic, foamy Kupffer cells and appeared rather basophilic. The changes in the spleens were similar but more widespread. The spleen (Fig. 7) was similar in some places to the spleen in visceral xanthomatosis. In a few places in the lung it appeared as if the septal cells had also become foamy. The kidneys appeared normal.

The amount of glycogen in the liver cells was minimal. No unusual material could be found in the organs by the use of the ethanolic PAS methods. For the sake of convenience, the main differences in the groups of mice are summarized in Table 2.

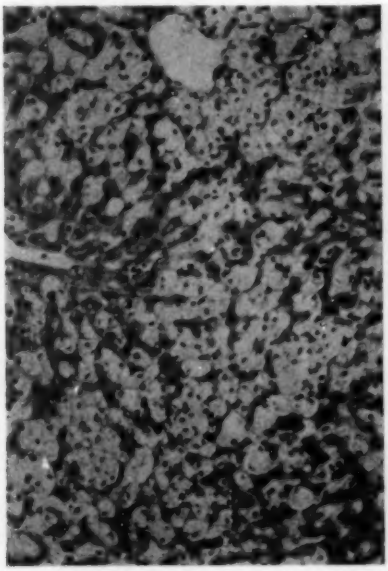

Fig. 6.—Mouse given twice daily 15 mg. of native levan for seven days. Liver. Extreme swelling of Kupffer cells, which appear vacuolated and foamy. Compression of liver cells. × 150.

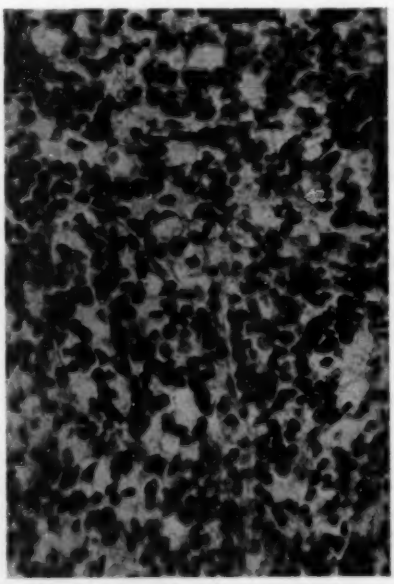

Fig. 7.—Mouse given twice daily 15 mg. of native levan for seven days. Spleen with foamy change of histiocytes. × 370.

TABLE 2.—*Different Responses of the Organs to the Injection of Various Polysaccharides*

	Liver Cells		Kupffer Cells	Splenic RES	Presence of Polysaccharides Granules in Organs
	Appearance	Glycogen			
Clinical dextran	Normal	Little	Normal	Normal	Demonstrated
Native dextran	Compressed, basophilic	Little	Foamy	Foamy	Demonstrated
Clinical levan	Swollen	Much	Normal	Normal	Not demonstrated
Native levan	Compressed, basophilic	Little	Foamy	Foamy	Not demonstrated
Control	Normal	Little	Normal	Normal	Not demonstrated

Comment

It is obvious from the findings reported above that there are marked differences between the levans and the dextrans, as well as between the high and the low polymers of each in their effect on mice.

The high-polymer polysaccharides elicit the foamy changes in the reticuloendothelial system in the spleen and liver which are common to many macromolecular compounds.[6] The reticuloendothelial-system cells swell greatly and possibly phagocytose the large macromolecules. On the other hand, while there is morphological evidence of phagocytosis in the case of the native dextran (presence of dark-purple granules in the sections stained by the ethanolic PAS procedures), no such evidence could be found in the case of native levan. This may point to the possibility that the levan, if ingested, may be changed by the phagocytic cell.

The low-polymer neutral polysaccharides seemed to cause very slight changes in the reticuloendothelial system. Two differences were noted between the comparable levans and dextrans. While the dextran could be demonstrated histochemically as extra- and intracellular granules, the levan could not. Secondly, the administration of levan provoked an increase in the liver-cell glycogen, whereas the administration of dextran elicited no changes in the liver cells. It should be noted, though, that the term "liver glycogen" is used here somewhat loosely: The compound accumulating in the liver cells of mice treated with clinical levan could not be distinguished histochemically from glycogen, although other proofs would be needed before it could be accepted definitely as being glycogen.

The differences between the levanized and the dextranized animals (both with high and with low polymers) indicate a different metabolic fate for the two classes of compounds. Levans seem to be sooner metabolized, changed, or excreted, whereas dextrans seem to remain for a longer time in their original state, or else in a state in which their histochemical characteristics remain unchanged. As the possible use of "clinical levan" as a substitute for "clinical dextran" in plasma expansion is being studied by the Laboratory of Microbiological Chemistry of this medical school, it should be noted that no pathological changes were observed in the animals treated by this compound. The problem whether, in view of the different behavior of the two compounds, clinical levan might not be a better plasma-volume expander than dextran cannot be answered on the basis of this study.

The doses of polysaccharides used in these studies were high. For a man weighing 80 kg. they would have corresponded to the daily administration of 2 liters of a 6% polysaccharide solution for seven consecutive days.

Summary

Changes in the internal organs were observed in mice given large doses of partly hydrolyzed and native dextrans and levans. The administration of the high polymers caused a foamy change in the reticuloendothelial cells in the liver and spleen. The administration of high-polymer or clinical dextran resulted in the extra- and intracellular deposition in the spleen, liver, kidney, and lung of a material having the

histochemical characteristics of the dextrans. No such depositions could be observed in the animals treated by high-polymer or clinical levans. In the liver cells of mice treated with clinical levan a marked increase in glycogen (or a compound indistinguishable from glycogen by the histochemical means used) was noted.

The evidence presented is believed to suggest a different metabolic fate for levans and dextrans, the levans being apparently the quicker to be metabolized, changed, or excreted. The possible superiority of levan over dextran as plasma expander is envisaged.

Miss F. Aslan and Mrs. J. Tarabulus gave technical assistance, and Mrs. H. Weinman did the photographic work.

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Electron Microscope Studies of Erythrocytes from Suckling Hamsters

Studies on Cells from Animals Exposed to the Chang Strain of the Common Cold Virus

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SING CHEN CHANG, Ph.D.
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The "common cold" (acute coryza, acute rhinitis) is attributable to infection of the upper respiratory tract with a virus. The symptoms and signs indicate the presence of an acute, but transient, alteration in the physiology of the mucous membrane of the upper respiratory tract, particularly that lining the nose and paranasal sinuses (Horsfall,[1] 1952). All of the etiologic factors are not yet completely understood, but there is good evidence indicating that one or more viruses may be involved in a "cold."

Kruse[2] (1914) and Foster[3] (1916) successfully transmitted colds experimentally to man by means of filtered materials. More than 10 reports have confirmed their findings, while 5 others have failed to confirm them. Dochez and associates[4] (1930) first reported the successful transmission of the common cold to chimpanzees by means of filtrates. Dochez and colleagues[5] (1931) reported the cultivation of the virus in tissue culture medium, and Kneeland and co-workers[6] (1936) described the cultivation of the agent on the chorioallantoic membrane of the chick embryo. Pollard and Caplovitz[7] (1947), Topping and Atlas[8] (1947), and Ward and Proctor[9] (1950) described cultivation of the virus in the allantoic sac of embryonating chicken eggs. Andrewes* (1949, 1950) has been unsuccessful in attempts to cultivate his strains in the chick embryo. All mammalian species tested, except man and chimpanzees, appear to be insusceptible to infection with the etiologic agent (Andrewes[10]). Colds experimentally induced by the intranasal inoculation of bacteriologically sterile filtrates or chick embryo passage material closely simulate natural colds and show all the features of the naturally acquired disease.

Recent studies by Andrewes[10] (1949) with collodion membranes of graded porosity indicate that the virus may have dimensions of 40μ-50μ or less. Andrewes'[11] (1950) results of tests for immunity to reinfection suggest that resistance may develop and persist for three or four months.

In a previous study (Reagan,† 1954) by electron microscopy of filtered infected nasal and throat washings from an adult affected with a common cold, spherical virus-like particles with a diameter of 80μ-90μ were observed. This virus was capable of inducing a cold in one chimpanzee. These virus-like bodies were not observed in normal nose and throat washings.

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From the Virus Department, Live Stock Sanitary Service Laboratories, University of Maryland, College Park, Md., and the Gastrointestinal Section, Walter Reed Army Medical Center, Washington, D. C. Professor of Virus Diseases (Major Reagan). Chief, Gastrointestinal Section, Walter Reed Army Medical Center, and Consultant in Gastroenterology to the Surgeon General (Lieutenant Colonel Palmer). Virologist (Miss Yancey). Assistant Professor of Animal Virology (Dr. Chang). Professor and Director of Live Stock Sanitary Service (Dr. Brueckner).

* References 10 and 11.

† References 12 and 13.

Materials and Methods

Nasal and throat washings were obtained three days after the onset of a common cold from the fourth suckling-hamster passage (Reagan [14]). These suckling hamsters (age 9 days) showed cold virus symptoms—running nose and wheezing, and the nostril area swollen, boggy, and inflamed (Reagan and associates [14]). Nasal and throat washings were likewise obtained for the control from suckling hamsters that had been exposed three days previously with isotonic saline. The infected nasal and throat washings from the infected suckling hamsters and those from the control (normal) suckling hamsters were filtered through a Type ST size L3 Seitz filter. The filtrates were then subjected to centrifugation and refrigeration in a Spinco ultracentrifuge for two hours at 31,410 rpm. The temperature of refrigeration in the outer jacket stayed constant at —10 C during the centrifugation. After the two-hour centrifugation, the supernatants from the specimens were discarded, and the sediments from each were resuspended in 1.0 cc. of chilled isotonic saline. Several drops of each specimen were placed on several Parlodion (collodion) film supports, which had been prepared 48 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff,[15] 1956) at arc tangent 4/12 and examined under the RCA electron microscope, Type EMU. The remainder of the infected washings was instilled nasally into eight suckling hamsters (immune to the cold virus) and into six suckling hamsters not immune. Five to six days after exposure the immune suckling hamsters appeared normal, showing no respiratory symptoms, while the nonimmune suckling hamsters showed cold symptoms (running nose and wheezing, and the nostril area was swollen, boggy, and slightly inflamed).

Observations

Upon electron microscopic examination of the concentrated suspension from the infected nasal and throat washings, uniform virus-like particles were observed. They were present in 70% of the 2000 microscopic grid fields examined.

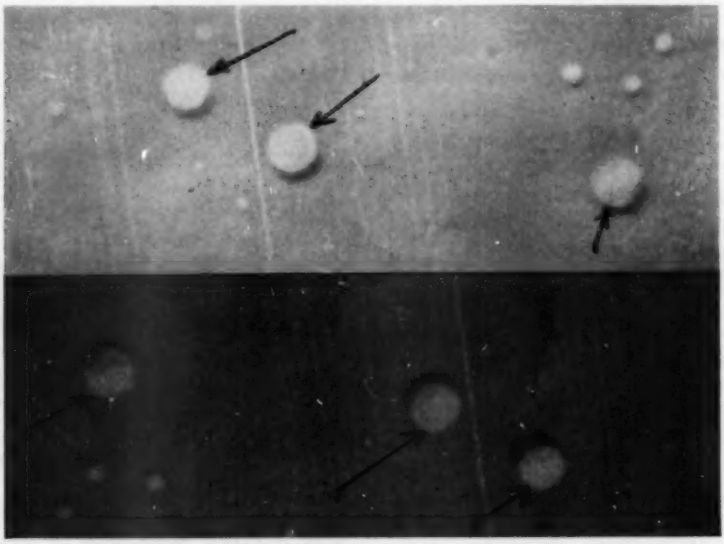

Fig. 1.—Electron micrograph of a common cold virus (MR strain) isolated from fourth suckling-hamster passage, shadowed with chromium at arc tangent 4/12; × 60,000.

Figures 1 and 2 are electron micrographs showing these virus-like particles. These particles are spherical in shape, with some "doughnut forms," and measure 90μ to 100μ by direct measurement. These bodies resemble the virus of influenza, described by Stanley (1946); the virus of measles, described by Reagan and associates (1952), and the cold virus, described by Reagan and associates ‡ (1954). No virus-like particles were seen in the concentrated normal nose and throat washings examined as above.

‡ References 12 and 13.

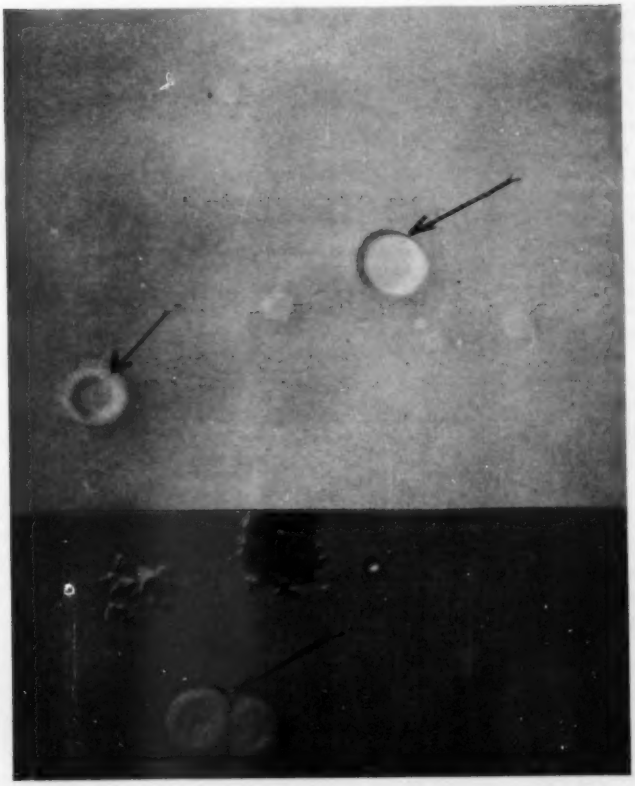

Fig. 2.—Electron micrograph of a common cold virus (MR strain) isolated from fourth suckling-hamster passage showing "doughnut"-shaped forms, shadowed with chromium at arc tangent 4/12; × 60,000.

Summary

Studies by electron microscopy of infected nasal and throat washings from the fourth suckling-hamster passage of a common-cold-virus strain showed this virus strain to be spherical and some to have "doughnut" shapes. The virus particles have a diameter of 90μ to 100μ by direct measurement. None of these bodies were found in normal nose and throat washings from suckling hamsters. The virus in infected washings was infectious for suckling hamsters. However, it had no effect on suckling hamsters having recovered from common cold symptoms described above, and thus presumably immune.

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News and Comment

ANNOUNCEMENTS

Annual Meeting of American Public Health Association.—The 84th Annual Meeting of the American Public Health Association will be held in Atlantic City, N. J., Nov. 12-16. Further information about the meetings may be obtained from the headquarters of the American Public Health Association at 1790 Broadway, New York City.

Ralph D. Lillie Makes South American Lecture Tour.—Dr. Ralph D. Lillie, medical director, U.S. Public Health Service, and chief of the Laboratory of Pathology and Histochemistry at the National Institute of Arthritis and Metabolic Diseases, recently made a trip to South America under the sponsorship of the Pan American Sanitary Bureau of the World Health Organization. In the course of his trip he gave lecture courses in histochemistry in Buenos Aires and Montevideo and lectures at Santiago and São Paulo.

International Cancer Cytology Congress.—The International Cancer Cytology Congress will be held at the Drake Hotel, Chicago, Oct. 8 to 13, 1956.

This Congress will be sponsored by the American Society of Clinical Pathologists, the College of American Pathologists, the Intersociety Cytology Council, and the International Union Against Cancer.

The general theme of the Congress will be Exfoliative Cytology, and the program to be presented during this period will stress but not be devoted exclusively to the subject of Exfoliative Cytology in all of its various aspects and its relationship to various specialties of medicine.

During this period three of the sponsoring organizations will hold their annual meeting, as follows:

The College of American Pathologists the evening of Monday, Oct. 8
The Intersociety Cytology Council the evening of Wednesday, Oct. 10
The American Society of Clinical Pathologists the evening of Thursday, Oct. 11
A joint banquet of all organizations will be held on the evening of Tuesday, Oct. 9.

Changes in the Intramural Coronary Branches in Coronary Arteriosclerosis

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There are a number of factors which are considered important in arterio- and atherosclerosis, especially those which affect the coronary arteries, such as heredity, hypertension, and obesity, and those related to endocrine disturbances, such as steroid hormone relationships, sex, thyroid dysfunctions, diabetes, and gout. Today the role of cholesterol metabolism, however, receives most of the attention. While inflammatory factors in the past have been considered as possible causes, the weight of evidence seems to favor an abnormal cholesterol metabolism as of prime importance. In this paper the old concept of inflammation as at least one factor in the causation of coronary arteriosclerosis will be discussed. This will be done not so much from the point of view of demonstrating actual inflammatory changes in the main coronary vessels as by a study of small intramural coronary branches, which may show residua of changes that can be interperted as of inflammatory origin.

In a previous study it was pointed out that the incidence of coronary arteriosclerosis and resulting myocardial changes in the younger age group was almost twice as great during the last 15 years as during the two immediately preceding decades.[1] The present investigation concerns itself with an examination of the myocardium in instances of arteriosclerosis and atherosclerosis of the large coronary arteries, with special reference to the state of the smaller intramural branches of the coronary arteries. This was done to determine whether or not residua of inflammation or other changes can be detected there that may give an indication of the nature of the disease in the large coronary arteries, which appears as primary arterio- or atherosclerosis. This investigation seemed pertinent because in certain instances of thromboangiitis obliterans[2] and also in old periarteritis[3] of the small ramifications of the coronary arteries in young people, the large coronary arteries were found to show typical arteriosclerosis with or without atheromas. It was thought that the latter signified perhaps end-stages of inflammatory conditions which were still recognizable in the smaller coronary branches, or that in some way old inflammatory lesions there might make the large coronary arteries more susceptible to atherosclerosis.

The assumption that coronary arteriosclerosis is somehow related to inflammation has received much attention in the past. As early as 1899[4] Heller spoke of "syphilitic arteriosclerosis," differentiated for the first time syphilitic aortitis ("syphilitic arteriosclerosis") from arteriosclerosis, and thus pointed out a relationship between inflammation and arteriosclerosis. Klotz, in 1913,[5] associated arteriosclerosis lesions with rheumatic fever. Boyd[6] assumed an inflammatory basis for coronary thrombosis. Karsner and Bayless[7] found inflammatory lesions in the coronary tree in all of the 56 rheumatic hearts examined. Von Albertini[8] differentiated between genuine arteriosclerosis

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From the Department of Pathology, Michael Reese Hospital.

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and secondary arteriosclerosis. The latter, he believed, is the result of inflammatory changes and is the type more frequently encountered in the coronary arteries of young persons, in so-called "juvenile" arteriosclosis. In some of his cases an outspoken angiitis obliterans was found to be the cause of secondary arteriosclerosis. To differentiate this lesion from genuine coronary arteriosclerosis, he advocated the term "arteritis stenosans coronaria." Von Albertini also maintained that it is only a question of time when the inflammatory arteritis, by the production of fibrosis and hyalinization with fatty depositions, changes into what he called secondary arteriosclerosis. Also, Bredt [9] concluded that thromboangiitis obliterans of the coronary arteries was the underlying factor in fatal juvenile coronary arteriosclerosis in seven instances he had observed. More recently, however, only a few references suggesting inflammation as one of the factors in arteriosclerosis, and especially coronary arteriosclerosis, are found. Thus Spain and associates [10] mentioned only one instance of inflammation of the coronary arteries in males under the age of 46.

In other locations, intimal fibrosis and hyalinization, changes which per se are characteristic of simple arteriosclerosis, are often seen in smaller arteries and are usually considered the result of inflammatory changes. Thus, endarteritis deformans is considered to be the result of chronic productive inflammation. These are the types of arterial change often encountered in the interlobar and arcuate arteries in the kidney associated with chronic pyelonephritis. Similar vessel changes also occur in other organs as a result of chronic inflammation close to the vessels.

Present Investigation

The purpose of this study was to determine whether, in certain instances, what appears as primary coronary artery sclerosis may actually represent the end-stages of an inflammatory condition. However, since it was felt that the main coronary arteries might no longer show the pathogenesis of these lesions, the intramural branches of the coronary arteries were examined in a relatively younger age group, to see whether or not remnants of inflammation might be detected in these vessels.

Although it had been planned to study the myocardium in instances of coronary arteriosclerosis in patients up to the age of 40, it was thought more profitable to include also patients up to 50 years of age. This group was decided upon because it was thought that remnants of inflammatory changes were more likely to be found in the small branches of the coronary arteries in a relatively younger age group, where it would seem that the time interval between the inflammation and what appeared as primary arteriosclerosis would be shorter—if the former had any bearing at all upon the latter. Besides, the increased incidence of coronary arteriosclerosis which we have observed during the last 15 years occurred especially in the age group of 40 to 50.

This study is based on microscopic examination of the myocardium in 100 patients. Only those cases were chosen which in the final autopsy diagnosis was listed as coronary arteriosclerosis or coronary atherosclerosis, with or without myocardial changes. Instances of minimal arteriosclerosis were not included. There were 32 females and 68 males. One patient was in the group between 11 and 20 years old; 7 were between 21 and 30; 18, between 31 and 40, and 74, between 41 and 50. Routinely, the hematoxylin and eosin stain was employed. When deemed necessary, the Van Gieson stain and the orcein stain for elastic fibers were also used. Special attention was given to the state of the intramural branches of the coronary arteries, and an intensive search was made for changes which could be interpreted as inflammatory in nature. Thus, perivascular cellular infiltrations, perivascular or adventitial fibrosis, intimal fibrosis, the arteritis obliterans type of lesions, and possibly also fibrous changes in the media were looked for in particular, since they all may indicate preexisting arteritis of any type. Perivascular foci of inflammatory cells consisted principally of lymphocytes, a few large mononuclear cells, and an occasional myocardial reticulocyte and mast cell. If infarcts or areas of fibrosis were found in the same heart, a search for vessel changes was made remote from the area of infarction.

Intimal fibrosis and also hyalinization was often encountered, but was frequently isolated and knob-like, causing an appreciable narrowing of the lumen in only 22 cases. Proliferation of intimal endothelial cells alone was also seen, but thrombi were not present. The internal elastic lamella was often thickened, but splitting or multiplication of the lamella was not observed. Periadventitial fibrosis was very common. In these instances the adventitia, even if also fibrosed, could be distinguished from perivascular fibrosis. In about one-fourth of these cases varying numbers of lymphocytes were also found. In 15 cases the perivascular tissue was lamellated, thus producing a characteristic "onion-skin" mantle about the vessel.

Other changes manifested themselves in the form of adventitial fibrosis and thickening of the media, which resulted in an apparent increase in the width of the media and adventitia. The fibrous changes in the media and adventitia were recorded as 1+, 2+, and 3+. M 1+ refers to a thickness of the media exceeding one-third of the total diameter of the artery, and M 2+, to a thickness of half the diameter. As far as the width of the adventitia was concerned, considerable variations were encountered under normal conditions, obviously depending upon the various locations. The adventitia often merged with, and was inseparable from, the interstitium in most places. The closer to the epicardium the coronary branch was located, the wider was the adventitia. In samples where the adventitia was considered normal, it was represented by one narrow rim of collagenous tissue measuring about one-fifth the thickness of the media, or slightly less, depending on the location. The thickness of the adventitia was considered abnormal if the ratio between the adventitia and the media (A/M ratio) exceeded 1. A 1+ refers to an A/M ratio of about 1.5/1; A 2+, to an A/M ratio of 2/1. An excessive A/M ratio and also excessive fibrosis of the adventitia were taken as evidence of old inflammation, especially since in a number of cases various inflammatory cells were found in the adventitia and transition could be observed from perivascular infiltration to perivascular fibrosis.

Table 1 lists the respective changes encountered in the various sections. It should be pointed out that the changes in the adventitia, media, and intima are listed separately, even though they may have occurred in the same heart. While, thus, various lesions were found 172 times, they were actually seen in 82 cases. This is indicated in Table 1 by the number of cases showing no remarkable changes.

Seventeen of the cases in the age group of 41 to 50 showed A 1+ lesions, and 16 cases in the same group revealed lesions graded as A 2+. A 3+ lesions were found in only two cases. These patients had histories of repeated myocardial infarcts. In the same age group, 19 cases showed medial thickening graded as M 1+ and 6 cases, as M 2+. In the 31-40-year age group, eight cases showed a A 1+ thickening of the adventitia and five cases were

TABLE 1.—*Summary of Arterial Changes Found in the Intramural Branches of the Coronary Arteries in the Various Age Groups*

Ages, Yr.	Media		Adventitia			Intimal Fibrosis	Perivascular Fibrosis	Perivascular Infiltration	No Remarkable Changes
	+	++	+	++	+++				
11-20						1		1	1
21-30	1	2	3	3		2	4	4	
31-40	4	4	8	5		8	9	10	1
41-50	19	6	17	16	2	16	15	10	16

graded as A 2+. Of the 18 cases in this age group, 4 showed medial thickening exceeding one-third the arterial diameter, classified as M 1+, and 4 were graded as M 2+, in which the thickening approached one-half the arterial diameter. Three of the seven cases in the age group of 21 to 30 showed an A 1+ grading, and three others revealed A 2+ lesions. One case was classified as M 1+ and two as M 2+. Intimal fibrosis was encountered 27 times and perivascular fibrosis 28 times. Recognizable evidence of perivascular infiltrations was seen in 25 instances. Eighteen cases showed none of these changes.

It thus seems clear that in 82 out of 100 patients with arteriosclerotic changes in the large coronary arteries, changes were also found in their intramural branches. These were characterized by thickening of the media and adventitia. Intimal fibrosis of small coronary branches and foci of inflammatory cells were found in 21 of these cases. In 32 of the 82 cases the perivascular fibrosis with perivascular infiltrations was severe enough to suggest an old periarteritis, particularly since, in some of these, perivascular infiltrations of lymphocytes were marked and a few polymorphonuclear leucocytes were demonstrable. Intimal fibrosis was seen in 27 of these cases.

There are several types of arteritis which in their healed stages are characterized principally by perivascular fibrosis and medial fibrosis, with or without intimal changes. Foremost among these are rheumatic arteritis; periarteritis nodosa, or polyarteritis; nonspecific arteritides, as seen secondary to various acute infectious diseases, and arteritis in hypersensitivity. To determine whether any evidence of hypersensitivity had been present which might have been responsible for blood vessel changes, the histories and autopsy findings of these 82 patients were carefully reviewed for possible pertinent data.

Analysis of the histories was difficult. The clinical data were usually deficient in recording past illnesses. In some cases the patients were admitted through emergency service and only a very brief history was obtained. In most of these emergency admissions the history was given by an informant, with questionable accuracy. In other cases the physicians in charge of obtaining the history might have concentrated their attention mostly or only on the facts that were apparently more related to the present illness, and the past illnesses might easily have been neglected. Further, in the majority of the cases, the records failed to mention the minor common infections, such as sore throat, common cold, and other slight respiratory or gastrointestinal disturbances, which may have caused seemingly unimportant allergies. Yet a review of the clinical records disclosed that 12 patients had histories suggestive of old hyperergic reactions (at least to judge from certain "allergies" the patient had) or that

TABLE 2.—*Clinical Evidence of Previous Hyperergic Reactions*

Sex & Age	Statement as Given in Clinical Records Concerning Possible Hyperergic Reaction	Changes in Intramural Coronary Arteries
M 33	Bullous lesions following administration of adhesive tape*	A +, M +
M 29	Reaction following sulfonamide drug administration	A + +, perivascular infiltrations
M 27	Reaction following sulfonamide drug administration	A + +, intimal fibrosis
M 36	Reaction following sulfonamide drug administration	A +, intimal fibrosis
F 48	Skin rash after unknown liquid medicine	A +, perivascular fibrosis, intimal fibrosis
M 46	"Long history of allergy"; reaction following penicillin administration	M +, intimal fibrosis
F 47	Intolerance of fat and green*	Perivascular fibrosis
F 42	Allergic thrombocytopenic purpura	A +, perivascular fibrosis
M 40	Eruptions after hexamethonium	Perivascular infiltrations
M 48	Allergic to barbiturates	A + +, perivascular fibrosis
M 40	"Asthma"	Intimal fibrosis and perivascular infiltration
M 50	Allergic to penicillin	A + +, perivascular fibrosis, intimal fibrosis

* These 2 cases presented some controversy as to whether they actually represented hyperergic reaction. The bullous lesions resulted from applications of the adhesive tape and could well be a physical irritation rather than hyperergic reaction. "Intolerance of fat and green" was a subjective statement from the patient herself, and not a diagnosis.

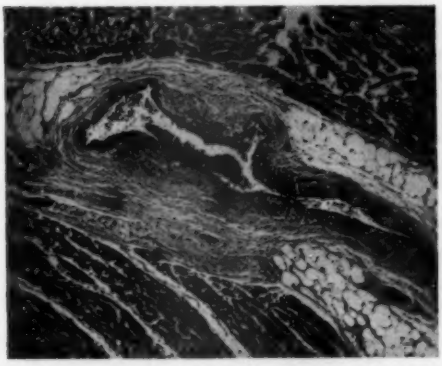

Fig. 1.—Intramural branch of coronary artery. Note the perivascular fibrosis and intimal thickening. Taken from a patient with a history of hypersensitivity. Hematoxylin and eosin stain; reduced to 73% of mag. × 100.

Fig. 2.—Note the "onion-skin" perivascular fibrosis. There is also some intimal fibrosis. Taken from a patient with a history of hypersensitivity. Hematoxylin and eosin stain; reduced to 73% of mag. × 125.

they were suffering from diseases considered allergic in nature. Table 2 is included to show clinical evidence of previous hypergic reactions.

There were 12 out of 100 patients with histories of hypersensitivity, which is known to cause arteritis or periarteritis. Two of the patients were under 30 years of age and two between 30 and 40.

Twenty-three patients had hypertension, and ten of these patients also had chronic pyelonephritis. Nine of these were females and fourteen males. All but five patients showed some degree of medial or adventitial changes in the intramural branches of the coronary arteries. Combined lesions of the media and adventitia were present in 10. Three showed medial lesions alone, and two presented only adventitial thickening. Intimal fibrosis was encountered in 6 of the 18 patients; perivascular fibrosis in only 2, and perivascular infiltration in 5. There were two patients who had had hypertension for 10 and 8 years, respectively, in whom no remarkable changes in the small coronary artery branches were found. In one patient showing M 2+ and A 1+, and in another with M 1+ and A 2+, the existence of

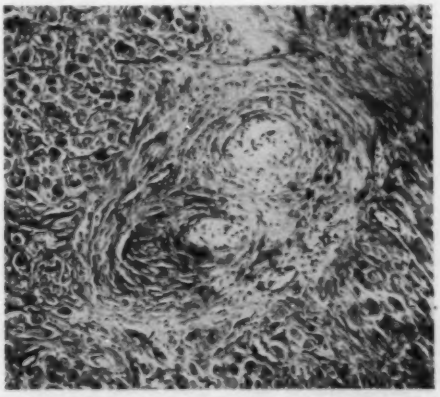

Fig. 3.—Intimal and periadventitial fibrosis with a few perivascular lymphocytes. Taken from a patient with a history of hypersensitivity. Hematoxylin and eosin stain; reduced to 94% of mag. × 100.

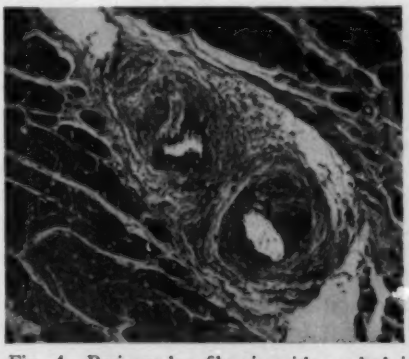

Fig. 4.—Perivascular fibrosis with marked intimal fibrosis. Taken from a patient with a history of hypersensitivity. Hematoxylin and eosin stain; reduced to 73% of mag. × 250.

TABLE 3.—*Changes in Intramural Coronary Arteries of Patients with Hypertension*

Sex & Age	Changes in Intramural Coronary Arteries	Duration of Hypertension, Yr.
M 33	M +, A +, perivascular infiltrates	6
F 48	Not remarkable	10
F 50	M +, A + +, intimal fibrosis	4
F 48	Not remarkable	"Years"
M 42	M +, A +, perivascular fibrosis	Not recorded
M 49	M +, Intimal fibrosis	8
M 49	A + +	3
M 48	M + +, A + +, perivascular infiltrates, intimal fibrosis	"Years"
F 47	M +, A +	9
M 39	M + +, A +, perivascular infiltrates	3
F 40	M +, A +, intimal fibrosis	8
M 50	M +, A +	5
F 38	M + +, perivascular infiltrates	More than 7
M 32	A + +, perivascular fibrosis, intimal fibrosis	14
M 46	M +	12
M 49	M +, A +	2
M 31	A + +, perivascular infiltrates	9
M 37	M +, A +	4
F 48	A + +, intimal fibrosis	16
M 47	Not remarkable	8
M 41	A + +	Not recorded
F 46	Not remarkable	4
F 46	Not remarkable	Not recorded

hypertension had been known for three and four years, respectively. The finding in this group are given in Table 3.

From Table 3 it would seem at first that hypertension per se may be responsible for the changes in the intramural branches of the coronary arteries, since, among our 100 patients with coronary arteriosclerosis in this age group, there were 23 with hypertension, and 18 of these showed pertinent changes. It is well known that arterial hypertension is often associated with coronary arteriosclerosis. Whatever the cause of this association, it may perhaps also hold for the changes in the intramural branches. That an inflammatory factor must also be considered seems particularly pertinent, since 5 of these 18 patients disclosed perivascular infiltration. The autopsy findings indicate that the hypertension in 10 of these patients was caused by the chronic pyelonephritis. It might be possible that the intramural coronary artery changes may have been the result of the chronic infection, since blood vessel changes occur in instances of chronic infectious diseases.[8] However, just why the branches of the coronary arteries or the main coronary arteries should be principally affected is not clear, although the continuous movement of these vessels in association with the heart beat may, in some way, be significant. A possible relationship to altered hemodynamics is considered below.

There were 7 patients with diabetes mellitus among the 100 cases studied. Four were females and three were males. All showed some histologic changes in the intramural coronary arteries. Four also showed intimal thickening. Medial lesions were found in three cases, and adventitial thickening was seen also in three. The findings in this group are indicated in Table 4.

TABLE 4.—*Changes of Intramural Coronary Arteries in Patients with Diabetes Mellitus*

Sex & Age	Changes in Intramural Coronary Arteries
F 31	Intimal thickening
F 50	Intimal thickening
M 38	Intimal thickening M +
M 42	M + +
F 46	Intimal thickening A + +
M 50	A + +
F 44	M +, A +

It is interesting that in those patients with diabetes mellitus changes were found not only in the main coronary arteries but also in their smaller ramifications. Morphologically, none of these changes suggested the presence of lipid substances in the intima. Unfortunately, the data on blood chemistry findings were insufficient and too few to warrant any statement in regard to the cholesterol content of the blood in these patients.

Table 5 is included to summarize changes in the intramural branches of the coronary arteries in instances of recent, recurrent, or old endocarditis.

In 10 of our cases with coronary sclerosis, autopsy findings disclosed some type of endocarditis. Outspoken rheumatic endocarditis with Aschoff bodies in the myocardium was found three times, while old endocarditis, most likely of rheumatic origin, was found in four additional instances. Changes in the intramural branches of the coronary arteries were encountered in all. There were intimal proliferation with fibrosis, perivascular fibrosis, and perivascular infiltrates, in addition to changes in the media and adventitia. Outspoken recent or older end- or periarteritis was seen

TABLE 5.—*Changes in Intramural Coronary Arteries in Cases of Endocarditis*

Sex & Age	Main Diagnosis	Changes in Intramural Coronary Arteries
M 33	Acute bacterial endocarditis superimposed on healed endocarditis	M +, A +, old endarteritic and periarteritic changes
M 42	Old endocarditis, rheumatic type	A + +, marked perivascular and intimal fibrosis
F 47	Old deforming endocarditis, rheumatic type	M +, A + +, marked intimal proliferation, perivascular fibrosis
F 47	Old deforming endocarditis, rheumatic type	M + +, A + +, severe perivascular fibrosis with marked intimal proliferation, perivascular infiltration with lymphocytes or large mononuclear cells
F 37	Old deforming endocarditis, rheumatic type	M + +, A + +, perivascular fibrosis and intimal proliferation
F 50	Acute nonbacterial thrombotic endocarditis	Perivascular fibrosis
M 42	Recurrent rheumatic endocarditis	A + +, marked endothelial intimal proliferation with perivascular infiltration of lymphocytic and myocardial reticulocytes and periadventitial fibrosis
M 50	Old endocarditis, rheumatic type	M +, A + +, perivascular infiltration and fibrosis
F 17	Acute and recurrent rheumatic pancarditis	A + +, severe end-and periarteritis
M 22	Acute and recurrent rheumatic endocarditis	M +, A + +, perivascular round-cell infiltration with foci of fibrinoid degeneration and fibrosis

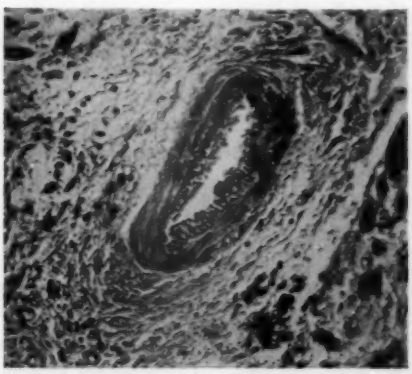

Fig. 5.—Marked periadventitial fibrosis with old periarteritis and the presence of a few large cells, suggesting an old Aschoff body. Taken from a heart showing old endocarditis. Hematoxylin and eosin stain; × 240.

in eight cases. The old periarteritis was characterized by concentrically arranged, laminated connective tissue about small arteries, characteristic of the so-called "onion-skin" lesion. The adventitia in five instances was also infiltrated with large mononuclear cells, small lymphocytes, and plasma cells, which occasionally also extended into the media. Often, too, the intimal fibrosis was nodular rather than diffuse. In short, these changes were quite characteristic of those described by Karsner and Bayless [7] and were thought to be typical of rheumatic arteritis.

There were also two additional cases where there was a history of rheumatic fever but where at autopsy changes were not encountered either in the heart valves or in the myocardium. However, intramural branches of the coronary arteries disclosed changes similar to those described above. Whether or not these are rheumatic or hyperergic in character could not be decided.

In summary, of the 82 cases in which changes in the intramural branches of the coronary arteries were found, there were 12 showing some evidence of hypersensitivity, 10 with endocarditis, 2 with histories

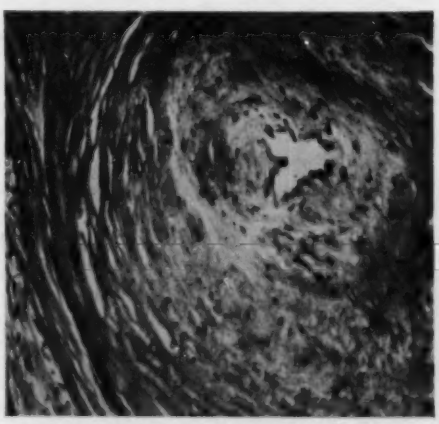

Fig. 6.—Marked periadventitial fibrosis and intimal fibrosis. Taken from a heart showing old endocarditis. Hematoxylin and eosin stain; reduced to 94% of mag. × 300.

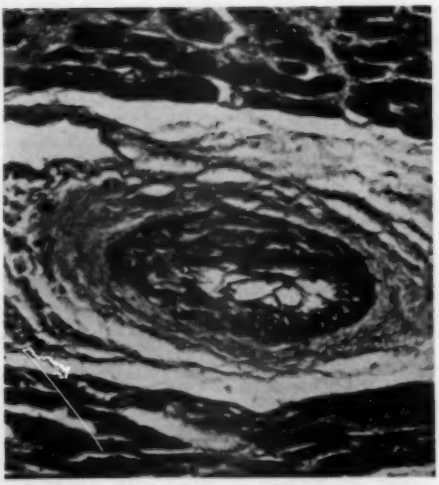

Fig. 7.—Marked adventitial fibrosis and intimal fibrosis. Taken from a heart showing old endocarditis. Iron hematoxylin and eosin stain; reduced to 94% of mag. × 240.

of rheumatic fever, 7 with diabetes, and 18 with hypertension. Although there were 23 patients with hypertension, only 18 of them showed the changes. There were 33 more cases which showed various changes in the intramural coronary artery branches, where there were neither endocardial lesions nor an obtainable history of allergy, hypertension, or diabetes. As stated, however, no pertinent questions had been asked in regard to a possible hypersensitivity. Thus, it is still possible that some of these patients may have had allergies. The microscopic lesions in at least 6 of these 33 hearts may well be explained on the basis of old periarteritic changes. In 10 other instances, nephritis or nephrosis was found at autopsy. These cases will be subjected to a more detailed study. In five instances there were a history and corroborating autopsy findings of long-lasting infectious diseases, such as chronic pulmonary or urinary tract infections. Thus, of the remaining 33 cases, 15 disclosed chronic infectious diseases or a history of these. In only 18 instances was there no evidence of chronic disease or of any other condition that is sometimes associated with coronary arteriosclerosis. Yet in six of these the microscopic changes were consistent with those of old periarteritic lesions.

Comment

Among 100 cases in a relatively young age group which grossly showed varying degrees of coronary artery sclerosis or atheromatosis, changes in the intramural branches of the coronary arteries were encountered in 82. These changes consisted of intimal proliferation, fibrosis and hyalinization, and medial and adventitial fibrosis with or without perivascular infiltrates. All of these changes are suggestive of old arteritis, and many cases still showed stigmata characteristic of periarteritis. Analysis of the autopsy findings and of the clinical histories disclosed that hypertension was present in 23 of the 82 patients, diabetes in 7, a clinical history suggestive of hypersensitivity in 12, evidence of recurrent or old endocarditis in 10, and a history of rheumatic fever in 2.

Cases Associated with Hypertension.—There were 23 such instances. In five the hearts showed no significant changes in the coronary artery branches. The principal changes were adventitial and medial fibrosis, but intimal fibrosis and perivascular changes were also frequent. In those cases in which it was noted in the clinical history, the duration of the hypertension was 2 to 10 years. There was no relation between the degree of the changes in the intramural branches of the coronary arteries and the duration of the hypertension. Perivascular infiltrations were found in five hearts. Intimal fibrosis with narrowing of the lumens of coronary branches was seen in six instances.

It is well known that hypertension is associated with increased incidence and severity of coronary atherosclerosis. As a matter of fact, Ernstene [11] found that about 50% of patients with coronary heart disease had coexisting hypertension. It may well be that in our cases the changes in the intramural coronary artery branches may also be somehow associated with the hypertension. While this may be true in a general

way, there were still five instances with outspoken evidence of old inflammatory disease in the coronary artery branches. In 10 instances hypertension was associated with pyelonephritis. There is no apparent relationship between such an infection and coronary arteritis, although the 5 cases which showed old arterial inflammatory changes were found among the 10 which showed chronic pyelonephritis. The possibility that the narrowing of branches of the coronary arteries may cause hemodynamic changes, with resulting inimal thickenings in the large coronary arteries, must also be considered. These intimal changes then may well form the initial change of arterio- and atherosclerosis (von Albertini's secondary arteriosclerosis). Baurle,[12] more recently, also found sclerosis in the smallest ramifications of the coronary arteries in instances of hypertension, with narrowing of their lumens. However, whereas in some of the cases he also noted marked accumulations of lipids in the thickened intima, we have seen no evidence of such lipids.

Cases with Diabetes.—There is an extensive literature on the association of arteriosclerosis and diabetes, and of coronary atherosclerosis and diabetes in particular. Among our 100 patients, there were 7 diabetics, all of whom showed changes in the intramural branches of the coronary arteries. Simple intimal thickening was found in four of the seven cases and was associated with medial thickening in one and adventitial fibrosis in another.

Fatty changes in the thickened intima were not encountered, but were frequent in the large coronary arteries. However, since evidence of inflammatory changes may be found in the coronary branches, it would seem possible that at least in some cases these changes may also be present in the large coronary arteries and, because of the altered cholesterol metabolism, predispose to subsequent atherosclerosis, provided, of course, that the alterations in the intramural branches can be used as indicators of the main coronary arteries.

Lesions in the Coronary Arteries Associated with Endocarditis.—As stated, in 10 cases evidence of endocarditis was found. Three of these were outspokenly rheumatic in origin and five were of rheumatic type, i.e., showing rheumatic stigmata, but no Aschoff bodies. Arterial changes in the branches of the coronary arteries in these instances have often been described[7] and are classified as rheumatic arteritis. The changes found in our cases correspond well with those described by these authors and others. As in Karsner and Bayless' cases, intimal changes in the coronary branches were much more frequent than would be indicated on gross examination. These changes in the coronary artery branches may predispose the large coronary arteries to fibrosis occurring early in life, as von Albertini suggested.[8] There were two additional cases with similar changes in the coronary artery branches where there were no changes in the heart valves, but where there was a history of rheumatic fever.

Lesions in the Coronary Arteries Associated with Hypersensitivity.—Twelve percent of our patients gave clinical histories suggesting hypersensitivity. In all of these, changes in the coronary artery branches were found, some of which could well be explained as the result of hypersensitivity. As stated, evidence of hypersensitivity was elicited only from the record, and no specific questions were asked in this respect. Thus, it might well be possible that in other cases where similar vessel changes were found, especially in six of our unclassified cases, direct questioning might have disclosed more such instances. It is noteworthy that 24% of our cases yielded a history and/or findings consistent with etiologic factors linked with causes of periarteritis: rheumatic fever and hypersensitivity. Interesting in this respect are studies by Grorud,[13] who stated that from the histologic picture in the early stage of coronary sclerosis, with fibrinoid swelling, etc., an allergic origin of at least some cases is possible.

Summary.—Among 82 cases which showed old lesions of the coronary artery

branches within the myocardium, hypertension, old endocarditis, or a history of rheumatic fever, some evidence of hypersensitivity or diabetes was found in 49. There were also five instances of hypertension which showed no changes in the intramural coronary arteries. Among the remaining 33 cases, 10, as stated, disclosed glomerulonephritis or nephrosis and 5 showed chronic infectious diseases. In the remaining 18 instances, with old changes in the intramural branches, there was no apparent etiologic factor. However, at least six of these showed microscopic changes which may well be explained on a hypersensitivity basis. It is possible that a careful and pertinent history of these patients might have likewise revealed some evidence of hypersensitivity. In only 12 of 82 cases which showed intramural coronary disease and gross coronary sclerosis and atherosclerosis were none of the diseases present which were found in the other instances, nor was it possible to elicit any history of hypersensitivity.

There is, thus, a group of cases affecting a younger age group where various changes are found in the smaller branches of the coronary arteries which can be interpreted as inflammatory in origin. Yet the large branches of the coronary arteries showed either arteriosclerosis or atheromatosis or both, but no evidence of inflammatory lesion. The possibility that the changes in the smaller arteries were coincidental can be ruled out because of the high percentage of cases (82%) in which they occurred. It might be possible that the lesions in the smaller arteries, perhaps because of resulting pressure changes in the large coronary arteries, are somehow instrumental in predisposing these vessels to intimal fibrosis, and later to secondary arteriosclerosis or atheromatosis, whether due to metabolic or to chemical alterations. However, we are intrigued by another possibility: It is well known that inflammatory changes or any pathologic processes close to blood vessels often cause intimal proliferation and subintimal fibrosis. Even though no evidence of inflammation was found in the larger coronary arteries, it might well be that the intimal changes there may be classed as secondary arteriosclerosis, in the sense of von Albertini,[8] and are remanants pointing to a past arteritis, evidence of which is still demonstrable in the intramural coronary artery branches. Adventitial fibrosis often accompanies coronary artery sclerosis of the large coronary arteries, and there is no way of distinguishing whether it is the result of coronary artery sclerosis per se or is perhaps brought about by another element. It should thus be considered that intimal and subintimal fibrosis of the large coronary arteries in some cases may be the result of an old inflammation, vestiges of which are still recognized in the intramural branches. It is noteworthy in this respect that one type of inflammation of the coronary arteries, rheumatic arteritis, had been thought to be somehow related to coronary sclerosis, and Karsner and Bayless, more than 20 years ago, pointed out that sclerosis of the coronary arteries is precocious in instances of rheumatic fever. Ten of our cases showed at autopsy an old endocarditis, which was considered rheumatic in origin in seven. Two additional patients gave a history of rheumatic fever. If rheumatic fever is a factor in the production of subintimal fibrosis and intimal changes in the larger coronary arteries, resembling, if not identical with, arteriosclerosis, it is likely that any other inflammatory lesions involving the coronary arteries may produce similar changes. Thus, any other inflammatory process causing endocarditis may cause such changes. Von Albertini [8] was particularly impressed with the relation of thromboangiitis obliterans to coronary arteriosclerosis. Our case of acute bacterial endocarditis also showed lesions in the intramural branches in the coronary arteries which in some instances were indistinguishable from those seen in rheumatic fever cases.

Rheumatic fever and other inflammatory processes which cause endocarditis, however, are not the only causes of inflamma-

tion of the coronary arteries. Rarely may coronary arteritis be seen in instances of infectious diseases. Common, however, are inflammatory changes in instances of hypersensitivity. The end-stages show only perivascular fibrosis, often concentrically arranged about the vessel, and various degrees of intimal or subintimal fibrosis. Remnants of inflammatory cells may or may not be present. Such changes with a history suggesting hypersensitivity were found in 12 cases. As stated, histories of hypersensitivity are difficult to obtain from routine clinical records and are noted only in exceptional cases. Yet suggestive evidence of hypersensitivity was found in 12% of all of our cases, and in 14.6% of those showing changes in the intramural branches of the coronary arteries. As in instances of rheumatic fever, it is likely that there were changes in the large coronary arteries similar to those in their intramural branches and that these changes eventually culminated in intimal and subintimal fibrosis with some adventitial fibrosis, indistinguishable from arteriosclerosis. These lesions probably also predispose to a deposition of lipids. Among others, von Albertini emphasized that intimal thickenings, whatever their cause, secondarily predispose to deposition of lipids and calcium salts, which changes then become indistinguishable from genuine atherosclerosis.

In a previous study, a distinct increase in the incidence of coronary arteriosclerosis in the younger age group was noted during the last 10 to 15 years, as compared with an immediately preceding 10-year period.[1] It may be more than a coincidence that during the last 15 years a number of chemotherapeutics and antibiotics have appeared on the market which are known to produce in some patients a hypersensitivity reaction. Thus, also, periarteritic lesions in the coronary artery branches may occur, with resulting changes in the large coronary arteries which are indistinguishable from primary arteriosclerosis. These may predispose to atheromatosis, similar to the vessel changes brought about by rheumatic fever.

Summary

In a study of the myocardium in 100 patients with coronary sclerosis and atheromatosis in the younger age group, remnants of inflammatory changes were found in the intramural branches of the coronary arteries in 82. These changes consisted of perivascular cellular infiltrations, perivascular or adventitial fibrosis, intimal fibrosis and hyalinization, the arteritis obliterans type of lesions, and fibrous changes in the media. In a number of instances, these changes were characteristic of old peri- or panarteritis. Autopsy findings and a review of the clinical histories disclosed evidence of endocarditis in 12 cases, in 8 of which it was most likely rheumatic in origin. Ten patients volunteered histories suggesting hypersensitivity or showed autopsy findings which are interpreted as allergic in origin. Six other cases showed changes in the intramural branches of the coronary arteries which were indistinguishable from those seen in the hypersensitivity group. The relation of the changes in the smaller branches of the coronary arteries to those of the large branches is discussed, and it is suggested that the intramural coronary artery disease may provide a clue to the nature of the changes in the large coronary arteries which are indistinguishable from primary arteriosclerosis and atherosclerosis. The question is raised whether perhaps the increase in coronary arteriosclerosis in the younger age group, as was observed during the last 15 years, may be associated with hypersensitivity related to the common use of antibiotics and chemotherapeutics.

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News and Comment

PERSONAL NEWS

Dr. F. K. Mostofi Lectures Before American Urological Association.—At the recent meeting of the American Urological Association, in Boston, Dr. F. K. Mostofi, of the Armed Forces Institute of Pathology, presented a three-hour illustrated lecture on the pathology of diseases of the genitourinary system.

Dr. Jean Oliver Awarded Addis Memorial Medal.— Dr. Jean Oliver has recently been awarded the second Addis Memorial Medal of the Los Angeles chapter of the National Nephrosis Foundation.

Books

Progress in Biophysics and Biophysical Chemistry. Volume 6. J. A. V. Butler, Editor. Price, $9.50. Pp. 274, illustrated. Pergamon Press, Ltd., 4-5 Fitzroy Sq., London, W. 1, 1956.

The presently reviewed volume of this generally valuable series contains seven papers of inhomogeneous quality.

A paper on protamines and nucleoprotamines, by Felix, Fischer, and Krekels, discusses the composition of these substances in certain fish sperm nuclei and gives information concerning other aspects of their chemistry. At the end of the paper there is mention of the genetic implications of the sperm-nucleus nucleoprotein content, which, from the point of view of intrinsic biological interest, seems disproportionately brief in relation to the importance of the problem.

Chromosome structure is discussed by E. A. Ambrose. A consideration of the chemical composition and physical structure of nuclear polymers is followed by remarks on the colloidal properties of nucleoproteins, leading up to the matter of organized chromosomal structure. There then follows a rather general discussion of current problems in chromosome research, which the reviewer found informative and stimulating.

Following these two papers concerning cell nuclei, the emphasis shifts, and J. Th. Overbeek writes about the Donnan equilibrium. The role of this type of equilibrium in the distribution of certain materials across cell surfaces and among phases within the cell, at both microscopic and submicroscopic levels, and as related to cellular transsurface potentials, has been a matter of concern for biologists for many years. This paper is well worth while in content, and also because it considers the theory of Donnan equilibria for nonideal systems. The loose thinking that often confuses equilibria with steady states is avoided. Remarks pertinent to the measurement of resting and action potentials are useful.

Next, there comes a discussion by Zamenhof of the very interesting transformation phenomenon. Since the nature of transformations, such as that of R pneumococci to S pneumococci by killed S pneumococci under certain conditions, is still obscure in many respects, the whole matter makes interesting and stimulating reading. The problems which still persist and the information which is available are clearly and ably presented in a sufficiently detailed fashion. The paper is a valuable addition to the literature.

The last three papers in this volume deal with neuromuscular transmission, with models for excitation processes, and with certain aspects of sense-organ physiology.

Neuromuscular transmission is discussed by B. Katz and del Castillo-Nicolau. Since Katz' laboratory has for some time been in the forefront of work in this area, it is to be expected that the review would be up-to-date, thorough, and authoritative. This expectation is fully realized. The paper considers practically all aspects of the problem of transmission at the neuromyal junction and can be recommended as rewarding reading for all who are interested in this field.

The paper on models for biological excitation processes by U. F. Franck is, for this reviewer, a difficult one to evaluate. It is always of interest to explore analogical systems, but it is probably only rarely that they contribute greatly to the understanding of their biological counterparts. For example, the so-called formal excitation theories of Hill, Rashevsky, and Blair provided precise statements of the rules of excitation but did little toward clarifying any of the physicochemical realities involved and failed to lead to any very revealing experiments. Similarly, the study of physical models has so far done little to clarify the problems of excitation. It may be that actual experimentation has not yet advanced to the point where it can take advantage of the model approach to the nerve problem.

The final paper of de Vries has to do with certain problems involving the physiology of sense organs. It is general, informative, and interesting.

In conclusion, it should be said that this volume is a welcome addition to the biological literature even if it is somewhat spotty in quality. A minor criticism may be offered concerning the technical editing; there are enough grammatical errors to be noticeable.

Diseases of the Chest. By H. Corwin Hinshaw, M.D., Clinical Professor of Medicine, and L. Henry Garland, M.D., Clinical Professor of Radiology, Stanford University School of Medicine. Price, $15.00. Pp. 727, with 634 illustrations, in 277 figures. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1956.

This textbook of diseases of the chest will prove of great value to students, house staff, and practitioners. The authors are to be congratulated on the logical organization of their work and on the very useful summary appearing in table form at the beginning of each chapter. The first one hundred pages of this book deal in a very concise and acceptable manner with physical, radiological, and laboratory diagnosis, with a chapter devoted to the anatomy of the tracheobronchial tree and a chapter devoted to pulmonary function. To some, the section on laboratory techniques will appear out of place in such a book, but it will undoubtedly prove useful to many. The chapter on laboratory technique does not deal completely enough with the problems of diagnosing fungus diseases, nor does it devote enough attention to the importance of exfoliative cytology of the bronchial tree. The clinical aspects of chest diseases are covered very thoroughly, but tuberculosis receives more attention than any other subject, comprising approximately 20% of the volume. The chapters on tuberculosis are exceedingly good and are worth the close attention of every student and physician interested in this disease. The chapter on bronchiogenic carcinoma could perhaps have been expanded, but it is still a very fine treatment of the subject. The book is written in a very readable style. The illustrations have been carefully chosen and are beautifully reproduced. The bibliography is extremely good, and appears as footnotes, with additional references at the end of each chapter. With each reference, the authors have given a concise summary of the content of the article. Drs. Hinshaw and Garland have indeed produced a most praiseworthy piece of work.

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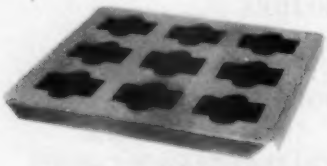

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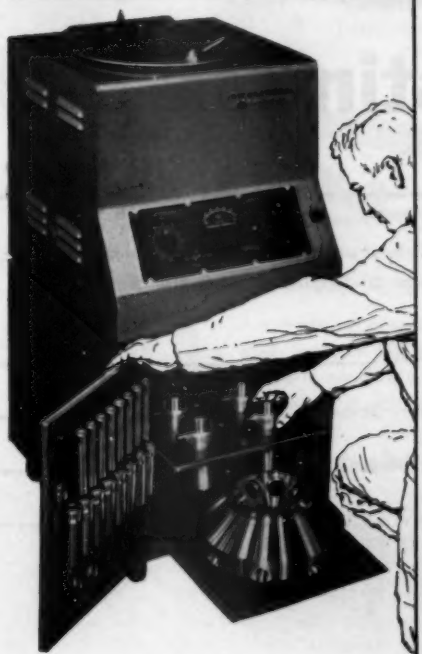

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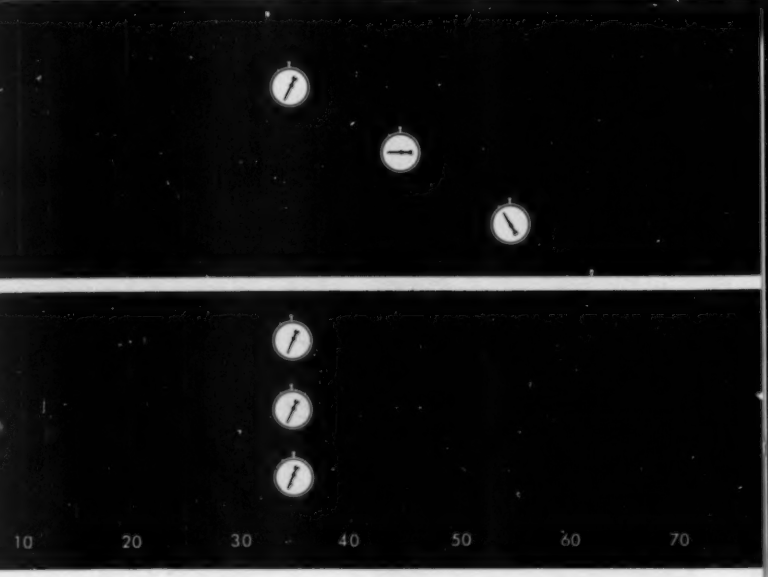

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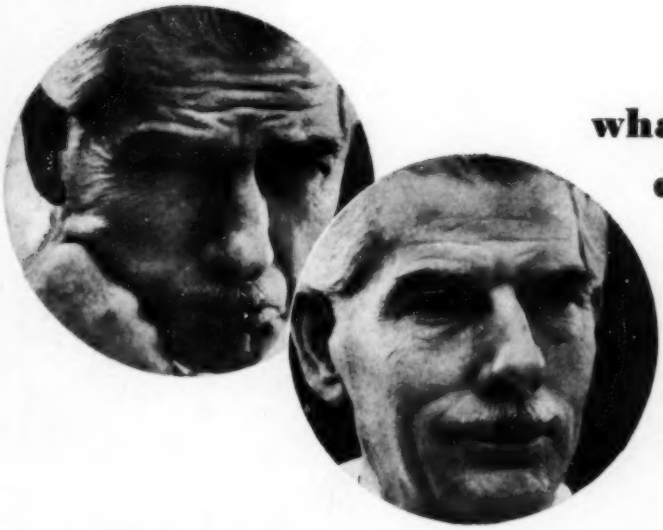

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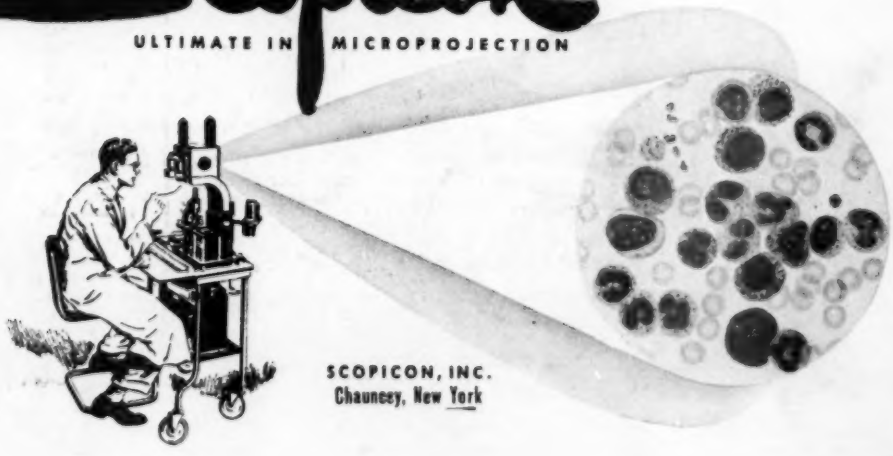

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